Important Dates and Deadlines:

- May 15, 2019: Scientific Session/CE Course Proposal Submission Deadline
- October 9, 2019: SOT Awards Nomination and Application Deadline for Most Awards
- October 18, 2019: 2019 Abstract Submission Deadline
- October 18, 2019: Undergraduate Diversity Award, Perry J. Gehring Diversity Student Travel Award, and Pfizer SOT Undergraduate Award Application 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 58th Annual Meeting of the Society of Toxicology, held at the Baltimore Convention Center, Baltimore, Maryland, March 10–14, 2019.

An alphabetical Author Index, cross-referencing the corresponding abstract number(s), begins on page 522.

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

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Scientific Session Types:

- CE Continuing Education Courses
- EC Education-Career Development Sessions
- IS Informational Sessions
- PL Platform Sessions
- PS Poster Sessions
- R Roundtable Sessions
- S Symposium Sessions
- W Workshop Sessions

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1001 SR01: Handling Uncertainties in Evaluating Mixtures: What’s the Difference between a “Similar” and a “Sufficiently Similar” Mixture?

S. C. Fitzpatrick, US FDA, College Park, MD.

Evaluating the safety and potential health risks from exposure to multiple chemicals, such as environmental chemicals, pharmaceuticals, consumer and personal care products, and pesticides and food contaminants, poses one of the major challenges for toxicological research and risk assessment. Significant advances have been made in recent years in better understanding and evaluating chemical mixtures. A key factor in risk assessments of chemical mixtures is the availability of reliable data on the identity, levels of exposure, toxico-kinetics, toxicodynamics, and toxicological interactions for the whole mixture or its individual components. Limited data or lack of data has a direct impact on uncertainty of the risk assessment of mixtures. As a result, risk assessment of chemical mixtures requires a lot of assumptions and uncertainty assessment. The commonly used risk assessment methods for chemical mixtures are whole mixture approaches and component-based approaches. The whole mixture approach is used when toxicological data are available for the mixture itself or toxicity data are available for a similar mixture or a sufficiently similar mixture that can be used as surrogate for the mixture of concern. This CE course will (1) provide an overview of challenges related to whole mixtures risk assessment and highlight approaches for evaluating sufficient similarity among related mixtures, and (2) present recent advances in safety assessment of complex mixtures using an alternative tiered approach, which utilizes in silico and in vitro approaches to identify safety data gaps and inform the need for additional studies. Attendees will be equipped to use similarity and sufficient similarity for whole mixtures, understand the assumptions, and understand how to address the uncertainties. This course would be of interest to scientists who conduct mixture risk assessment in different sectors, such as occupational health and safety, product safety, public health protection, or regulatory decision-making. This sunrise CE course complements the previous CE courses and sessions at SOT on mixtures and focuses specifically on the uncertainty assessment aspect of similar and sufficiently similar mixtures, which has not been discussed before.

1002 SR02: Publicly Available Exposure Tools to Inform the Toxic Substances Control Act

J. Wambaugh, US EPA/NCCT, Research Triangle Park, NC.

Exposure is a key component of chemical risk assessments, as highlighted by the recent amendment to the Toxic Substances Control Act mandating the US EPA to consider conditions of chemical use, as well as human and ecological exposures across the chemical life cycle. The US EPA Office of Research and Development has many ongoing exposure modeling efforts that may be informative for chemical safety decisions. This sunrise CE course covers how 21st-century exposure science tools could be used to inform chemical risk assessments. The first instructor will present a series of databases and models that are both peer reviewed and free to use. The second instructor will cover new, consensus exposure predictions for instances where minimal exposure data are available. Each lecture will provide examples that can be easily modified by course attendees for specific chemical risk assessment applications.

1003 AM03: Assay Development Principles and Good Research Practices for Rigor and Reproducibility in In Vitro Toxicology

M. Xia, NIH/NCATS, Bethesda, MD.

Toxicological research and testing heavily depends on the application of cell and molecular assays to provide mechanistic insight into the effects of chemical exposures as well as model systems to overcome the constraints of in vivo human and animal exposure studies. Despite being powerful tools, these assays are not immune from the "reproducibility crisis" that has cast a considerable shadow over all fields of biomedical research. Improving the rigor, reproducibility, and physiological relevance of both traditional and high-throughput cellular and molecular methods is critical to protect public health, increase the efficiency of drug and consumer product development, and ensure the reliability of data used in chemical regulation. Recent reports in both the scientific and public literature have revealed a need for increased rigor in preclinical research and highlighted experimental design, reagents (including antibodies and cell lines), and data analysis as key challenges to study reproducibility. The goal of this course is to provide participants with "good research practices" for the rigorous development, implementation, and interpretation of robust in vitro toxicological assays for reproducible results using physiologically relevant models. Presentations will follow a broadly applicable workflow, starting with the establishment of a verified cell culture model with increased physiological relevance. Participants will learn how understanding the nature of cells in vitro and treating cells as reagents can ensure the design of more reproducible assays. Strategies will also be shared for the successful implementation of high-throughput assays that enable the rapid and high-throughput assessment of both toxicity and efficacy using in vitro models with increased physiological relevance. This will be followed by global gene expression analysis using RNA sequencing, validation, and exploration of target gene expression with quantitative PCR, assessment of protein abundance, and post-translational modification using immunoassays, and evaluation of cumulative effects of exposures on cell physiology and viability. The final presentation will empower participants with the knowledge and tools to utilize innovative statistical measures that were developed specifically to enable reliable assessments about compound properties based on data from in vitro assays. This course will provide attendees with core principles and practices for widely used methods, which will facilitate the design and execution of a broad range of rigorous and reproducible experiments, increased throughput, and improved in-depth interpretation of data from both study findings and published literature. The content of this course will benefit researchers from industry, government, and academic labs who evaluate the safety of experimental compounds and wish to learn more about the latest models, methodologies, and analysis strategies.

1004 AM04: Complex Mixtures and UVCBs: Analysis, Testing, and Risk Assessment

C. V. Rider, NIEHS/NTP, Research Triangle Park, NC.

A complex mixture, as defined in a 2018 update to the Agency for Toxic Substances and Disease Registry Framework for Assessing Health Impacts of Multiple Chemicals and Other Stressors, has many chemicals (often of different chemical classes), has a composition which may not be fully characterized, and can arise from a single source or multiple sources. The related, but more specifically defined, term, UVCB substances (Chemical Substances of Unknown or Variable Composition, Complex Reaction Products and Biological Materials), has been applied by both the US EPA in the Toxic Substances Control Act (TSCA) Chemical Substance Inventory and the European Chemicals Agency (ECHA) under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulations. Complex mixtures and UVCBs can include foods and beverages, personal care or consumer products, reaction by-products, emissions, and leachates. They can exhibit a wide array of physicochemical properties and fall under different regulatory jurisdictions. However, there are common principles that can be applied to these substances to gain an understanding of their complex chemistry and evaluate their toxicity and/or safety. Historically, the prevailing dichotomy was to either treat these substances as single entities, thereby ignoring their complex and often dynamic nature, or apply a reductionist approach that only considered a small subset of known constituents (i.e., identified chemical constituents with available toxicity data). Progress in analytical chemistry techniques, untargeted analyses, and in vitro screening tools has allowed for a more comprehensive and holistic approach to complex mixtures. In this course, state-of-the-science approaches for evaluating complex mixtures and UVCBs will be presented. It will begin with a presentation of the regulatory challenges and views of complex mixtures from the perspective of the US FDA Center for Food Safety and Applied Nutrition (CFSAN). Next, recommended methods for chemically analyzing complex mixtures and identifying biological constituents will be presented. Untargeted approaches for assessing complex mixtures, such as metabolomics and chemometrics, will be addressed. The use of in vitro assays and alternative animal models in screening complex mixtures will be discussed, with attention on successful applications and pitfalls to avoid. Additionally, available methods and software for combining chemical and biological assay data will be presented. Finally, existing methods for comparing across complex mixtures and determining sufficient similarity of related mixtures will be presented. Presentations will address chemistry, biological activity, and the intersection of the two, with an intentional focus on how these data can be used in safety evaluations of complex mixtures. Throughout the course, speakers will provide terminology and definitions and highlight tools using a diverse array of examples, representing distinct categories of complex mixtures and UVCBs. This course will be useful to those interested in understanding complex mixtures from a product development, research, or regulatory perspective. Course participants will be provided with both big picture context on complex mixtures and specific, data-driven recommendations learned from application of the presented methods.
1005 AM05: Developmental Toxicity of the Skeletal System: Interpretation of Findings in DART Studies and Implications for Risk Assessment

M. R. Garry. Exponent, Seattle, WA.

Skeletal development represents a period of rapid patterning and specification of tissues that form the basis for subsequent growth in the developing organism. As a result, formation of the skeletal elements (e.g., bone and cartilage) is included as a standard endpoint in prenatal developmental toxicity studies. Abnormal findings are classified as variations or malformations; however, the interpretation of these findings and whether they result in functional deficits in postnatal life can significantly impact regulatory decisions. This presentation will highlight the diversity of use cases within each industry. The course will provide participants with an introduction to skeletal anatomy and physiology, that can facilitate the interpretation of abnormal findings from a toxicological perspective. Speakers from academia, industry, and government with expertise in the fields of skeletal biology and developmental toxicology will provide (1) a fundamental review of skeletal development in animal models currently used in developmental toxicity studies, with an emphasis on differences in developmental course and extrapolation between species; (2) a discussion of current and emerging methods to identify skeletal anomalies in prenatal and postnatal/juvenile developmental toxicity studies, and their relation to overall developmental toxicity, both in the animal models and their potential human relevance; (3) case studies to illustrate the concepts introduced by the first two speakers and specific challenges faced in the interpretation of study results; and (4) context from a regulatory perspective on the interpretation of abnormal skeletal findings and the evolving requirements needed to address skeletal toxicity concerns.

1006 Industrial Applications of Computational Toxicology in the 21st Century


Computational toxicology encompasses the development of computational models and computational tools applied to datasets of toxicological concern and the use of such methods for various applications. This is a wide field spanning hazard identification, prioritization for experimental testing, optimization of chemical space, and chemical risk assessment. These methods are used in many different industry sectors, such as consumer products, pharmaceuticals, and agrochemicals, as well as being widely used in the environmental sector and in governmental or regulatory organizations. The methods employed vary from simple to complex depending on availability and quality of data, and range from the application of structural alerts to machine-learning models of large-scale biological data and complex systems toxicology modeling. With increased pressure to reduce the number of animal experiments, accelerate the product development cycles, and lower costs, computational toxicology is a continuously developing area with yet-untapped potential. This course will provide participants with an introduction to the field, followed by a method section where different scenarios will be presented that guide the participants in how data are analyzed and models and tools are built, depending on the use case at hand, as well as data limitations. This will be followed by two presentations on practical applications of computational toxicology, the first one focused on consumer products (e.g., food, cosmetics) and the second on examples from the pharmaceutical industry. Both of these presentations will highlight the diversity of use cases within each industry. The course will end with a presentation discussing the regulatory landscape and examples of how such tools are used to support regulatory safety assessment of various products. The aim of this course is to introduce the discipline of computational toxicology to the nonexpert and provide the participants with a broad understanding of the many benefits of computational toxicology methods, as well as an understanding of the limitations and appropriate use of such methods for successful outcomes in an industrial setting. The learnings from this course are relevant for attendees from all industry sectors as well as from other research-directed organizations.

1008 AM08: Mechanistic Understanding and Quantitative Risk Assessment in Immunotoxicology

E. Corsini. Università degli Studi di Milano, Milan, Italy.

Considering the important health consequences associated with exposure to immunotoxic compounds, quantitative risk assessment in immunotoxicology is an area of growing interest. The discipline of immunotoxicology has refined several powerful tools to assess the safety of new drugs and other products. Novel approaches for assessment of hypersensitivity and cytokine-based assays to examine chemical-specific effects are moving the field away from the use of animals and providing a path forward for hazard identification and risk assessment. Although the majority of immunotoxicity studies are designed for hazard identification, there is a considerable amount of data demonstrating a threshold for both immunosuppression and contact sensitization exists, making quantitative risk assessment possible. The purpose of this advanced course is to provide guidance on how to perform risk assessment using immunotoxicology data. Following a brief introduction (first presentation), examples will be given for both immunosuppression (second presentation) and contact hypersensitivity (third presentation). In addition, to support animal-to-human extrapolation, mechanistic understanding is crucial and will be provided in this course (last two presentations). In 21st-century Toxicology, it is also crucial to integrate all information from in silico and in vitro methods into animal studies. Therefore, in a modern vision of immunotoxicology, integrated strategies will be described and examples provided in each presentation. This course will provide participants with the means and knowledge to conduct quantitative risk assessment using the effect on the immune system as the adverse outcome to protect humans from chemical-induced immunotoxicity and its consequences.

1007 AM07: Role of Toxicokinetics in Human Health Safety Assessments

S. Papineni. Dow AgroSciences, Indianapolis, IN.

Regulatory toxicity testing and risk assessment paradigms historically have been based on external doses, despite acknowledged scientific advantages in using systemic exposures. Integrating toxicokinetics (TK) into regulatory toxicity testing provides an opportunity to develop more relevant data by utilizing systemic dose in animals and predicted (modeled) or measured blood levels of chemicals in humans. This provides a foundation for improved evaluation of human relevance, life-stage susceptibility, mode-of-action or adverse outcome pathway, route-to-route extrapolation, and dose selection. In addition, there is increased emphasis on improving toxicity testing and safety assessment in alignment with the 3Rs principles of animal welfare (replace, reduce, refine), and expanded use and collection of TK information reduces the overall use of animals by eliminating unnecessary or redundant tests and provides more humane dose selections, which are less physiologically stressful on the animals. As with any scientific change in practice, the change in optimization of these approaches across the globe. Increased awareness and communication of the benefits of these approaches is key for global harmonization. This course aims to increase knowledge on the principles of TK and will enable students to explore the opportunities that TK offers to risk assessment (all stages: hazard identification, dose-response assessment, exposure assessment, risk characterization) and provide a forum for students to hear from scientists of varying backgrounds and sectors—regulatory, academic, and industry. There have been many advancements in technology and increased emphasis by regulatory agencies to collect TK data. However, the implementation and applicability of these data in regulatory toxicity testing have lagged considerably. The first talk will introduce the topic and present the basic principles of TK, and also will provide an understanding of why and when TK is useful for investigating issues in toxicology. The second presentation will review the experience of integrating and utilizing knowledge of TK in preclinical safety testing of pharmaceuticals. The third presentation will describe the standard testing protocols, technical details, and considerations to integrate TK in standardized guideline studies without use of additional animals and making the guidelines relevant to assessing risks to human health. The fourth presentation will provide a regulatory overview of integration of TK into various steps in the risk assessment process, using case studies to demonstrate how TK data have been used in pesticide risk assessment to improve the science underlying regulatory decision-making. Overall, this course will provide the needed background and approaches to implement TK, using practical examples that will enable the attendees to have a better appreciation of its utility in risk assessment and decision-making regarding chemicals for human health. This course also will highlight the shift toward utilization of high-throughput toxicity screening and nonanimal methods that both are in alignment with 3Rs principle and offer cost- and resource-effective means to prioritize chemicals. Thus, this course will be of a broad interest to testing laboratories, general toxicologists, and risk assessors across different sectors, including academia, regulatory agencies, and industry.

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Physiologically based pharmacokinetic (PBPK) modeling is widely recognized as a scientifically sound approach to characterize uncertainty in the quantitative relationship between internal and external exposures. The number of regulatory reviews of PBPK models has risen significantly in recent years to support decision-making regarding safety of environmental chemicals and pharmaceutical compounds. For environmental chemicals, PBPK modeling allows for extrapolations across species, life stages, and exposure routes/sequences of human biomonitoring measurements. For pharmaceutical compounds, PBPK modeling can be used to identify the need for dose adjustments in subpopulations, the potential for drug-drug interaction, and undesired pharmacokinetics properties such as low bioavailability or rapid clearance. The application of PBPK models to support regulatory risk assessment requires thorough vetting in the context of whether the model's performance is appropriate for its intended purpose. However, the growing list of applications and different acceptance criteria among agencies and across countries have increased the need for a more standardized approach to both model submission and review processes. To achieve such a goal, this course is designed to provide an overview of how PBPK models might be applied to investigate health outcomes resulting from exposures to environmental or pharmaceutical compounds, as well as to discuss the key elements being reviewed by different regulatory agencies. This overview will help to promote dialogue among developers, users, and evaluators of PBPK models across government, industry, and academia who seek to establish consistent model submission and review practices. In addition, this course provides training to both modelers and non-modelers, with the purpose of increasing the pool of potential peer reviewers for regulatory agencies so that they can conduct proper review of models in a timely fashion. To accomplish these training objectives, topics to be covered in this course include the principles of pharmacokinetics, fundamental concepts underlying PBPK modeling, data needs, and quality assurance during model development and implementation. The overarching objectives of this course are to highlight opportunities for harmonizing model submission and review processes and to increase the likelihood of model adoption at regulatory agencies. Disclaimer: The views expressed in this abstract are those of the authors and do not represent Agency policy or endorsement.

### 1009 PM09: Applications and Review of Physiologically Based Pharmacokinetic Modeling for Regulatory Risk Assessment


### 1011 PM11: Conducting Systematic Review in Toxicology—Why, When, How?

M. Wilks, University of Basel, Basel, Switzerland.

Systematic review is gaining interest in the field of toxicology, highlighted by regulatory requirements being globally instituted to conduct systematic review in support of safety assessments of chemicals and foods (e.g., via US EPA Toxic Substance Control Act [TSCA], US EPA Integrated Risk Information System [IRIS], and European Food Safety Authority [EFSA]). Systematic review refers to the objective and transparent process of collecting and synthesizing scientific evidence for reaching conclusions on specific research questions. While systematic review has been successfully used for decision-making in areas such as clinical medicine for many years, the implementation of systematic review within a toxicological context using established frameworks presents unique challenges. As such, several groups that conduct toxicological research have developed systematic review frameworks that take into consideration the breadth of data relevant to the environmental health and food safety sciences by extending and adapting the approaches developed for clinical medicine. This course will survey available approaches and tools for conducting systematic reviews in toxicology, provide information on the components and conduct of systematic review, and provide instructions on reporting and appraising systematic reviews. Particular emphasis will be placed on determining when a systematic review would be useful and how to determine the specific research question(s), critical appraisal of study quality for human and animal evidence, and structured integration of the evidence across evidence streams. Presenters will highlight and demonstrate tools and other software that can be used for study selection and screening, study quality appraisal, documentation, visualization, and decision-making. The course will provide the opportunity for participants to gain an understanding of why to choose to conduct a systematic review, when it is appropriate to do so, and how to conduct the critical elements of a systematic review, as well as gain an appreciation for the rigor and transparency that a systematic review requires (thus setting it apart from traditional narrative reviews). This course has strong relevance to toxicologists from diverse sectors, including researchers, regulators, risk assessors, consultants, and industry, who may need to use systematic review processes or even consider the results of systematic reviews in their practice.

### 1010 PM10: Beauty of the Skin Is in the Eye of the Beholder: A Basic Course on Dermal and Ocular Toxicology

M. F. Hughes, US EPA, Research Triangle Park, NC.

Every day we use our eyes to see what is going on in the world, while our skin provides key information to our brains by sensing the world around us through touch. Skin also protects our body by regulating our temperature. While the eyes and skin are two distinct organs, they have some commonalities. First, they both provide our bodies with a barrier to the external environment. Although the barrier properties of the skin and cornea are not impermeable or equivalent in their ability to protect, they provide a degree of impedance to physical assaults such as sunlight and xenobiotic penetration. Secondly, the outer anatomy of the skin and the eye are epithelial in nature, derived from the ectoderm. These two organs have differences in their physiology, functional purpose, toxicological response, and pathological outcome. Both organs are important to toxicology because they are exposed to the external environment but react differently to toxic insults than internal organs. The purpose of this course is to provide the audience with the fundamentals of dermal and ocular toxicology and methods to assess absorption and toxicity. The first presentation will focus on dermal anatomy and methods to assess dermal absorption. Factors that can affect dermal absorption will be discussed, as well as those learned from in vitro studies (e.g., static, flow-through methods) and in vivo methods to quantitate absorption. The strengths and weaknesses of these methods will be presented. The second presentation will emphasize dermal toxicity. An overview of the manifestations of dermal toxicity, its assessment biomarkers, and useful animal models of chemical-threat agents exposure will be presented. The third presentation will discuss ocular toxicity. The anatomy of the eye and manifestations of ocular injury and toxicity from a variety of drug and chemical classes will be presented. The fourth presentation will highlight toxicology of the cornea. The anatomy of the cornea, absorption of chemicals and drugs through this tissue, and implications of toxicity on the function of the cornea will be presented. The fifth presentation will cover advances in the field of nonanimal alternatives to toxicity testing for skin sensitization and ocular dermal irritation. Work to develop and validate integrated testing strategies and progress toward regulatory implementation will be discussed. Overall, by attending this session, the audience will gain basic information to understand the potential toxicological outcome of xenobiotic exposure to the dermal and ocular systems.

### 1012 PM12: Current Dose-Response Modeling Strategies and Applications in Chemical Risk Assessment

K. Shao, Indiana University, Bloomington, IN.

Quantifying dose-response relationships to evaluate the toxicity of environmental chemicals is a key step in human health risk assessment and has substantially evolved in recent years. The purpose of this course, to be delivered by a mix of groups of experts from government, academia, and industry, is to provide participants an overview of the currently prevailing dose-response modeling methodologies and tools with case studies and applications in chemical risk assessment. The first presentation will introduce basic concepts and terminologies of the benchmark dose (BMD) method, including discussions on the use of US EPA benchmark dose software (BMDS), how to model commonly available toxicological data, and how to interpret the results. The second presentation will discuss the categorical regression modeling approach, together with the US EPA Categorical Regression Analysis (CatReg) software and its application to chemical risk assessment. The third speaker will present how to apply the BMD methodology in a Bayesian framework to produce probabilistic estimates of interest (e.g., model parameter estimates, single model BMD estimates, and model averaged BMD estimates) to support probabilistic dose-response assessment. While the first three presentations complement each other regarding modeling methodologies, the last speaker will provide an overview to summarize the utilities of the strategies and tools through three case studies in the agrochemical industry to help participants reinforce the knowledge by using real-world relevance and experience.
The microbiome consists of indigenous microbial communities and the host environment that they inhabit. Current paradigm-shifting research indicates that the interaction between the host and the microbiome is an important regulator of many diseases and is changing the way that scientists think about the role microbes play in human health. The microbiome includes microbes that are both helpful and potentially harmful, and in a healthy individual, these microbial communities coexist without problems. However, when this balance is disturbed, dysbiosis can occur. One such factor that is emerging as a regulator of this balance is exposure to environmental pollutants that may perturb host-microbiome interactions to promote disease. The microbiome is a rapidly emerging field, and toxicologists from industry, academia, and federal agencies understand the importance of studying the impact of toxicants and pharmaceuticals on gut microbiome dysbiosis and host responses. However, approaching this vast area of study can seem daunting. This course is designed to provide practical information from experts in the field with the latest state-of-the-art tools so that toxicologists can incorporate the study of microbiome and host-associated responses into mechanistic research, risk assessment, and/or therapeutics. Following this course, participants will be familiar with current advances in microbiome research as it pertains to toxicology. An overview of experimental models and case study examples of microbiome toxicity and immunotoxicity will be presented. Further discussion on how xenobiotics change the microbial population and immune status of animals during developmental exposures will be provided. Concepts will be reinforced in a multigenerational toxicology case study that will take the participants through steps of experimental design, data collection, and reporting. The course will provide participants with practical knowledge and tools to conduct microbiome analysis using the metagenomics analysis server (MG-RAST). The latest information related to regulatory aspects for microbiome-based therapeutics approaches will be presented to participants. Overall, this course will provide a comprehensive overview of study design, data analysis, and challenges in biotherapeutics using examples of toxicant-induced intestinal microbiome dysbiosis.

Mitochondria are critical subcellular organelles, as they provide more than 95% of the energy for biochemical and physiological functions, in addition to playing a critical role in lipid metabolism, steroidogenesis, and programmed cell death. In the context of this course, both structural and functional features of the mitochondria will be addressed. Involvement of mitochondria in health and in drug-induced cellular and subcellular toxicities will be discussed, and the practical applications will be described. In the first lecture of this course, the prominent role of mitochondrial toxicity in adverse outcome pathways (AOPs) mechanistically describing a wide spectrum of organ-specific toxicities will be demonstrated. In the second lecture, the central role of mitochondria in drug-induced programmed necrosis and the impact of adaptive mechanisms such as autophagy and mitochondrial biogenesis on cell survival and regeneration will be highlighted. The third lecture will focus on evaluation of mitochondrial function by confocal and multiphoton microscopy, and measurement of respiration and glycolysis. In the last lecture, the metabolic capacity of mitochondria in terms of local reactive metabolite generation, as well as toxicological outcomes, will be discussed.

**Advances in In Vitro to In Vivo Extrapolation: Approaches and Applications**

N. Kleinsteuber, NIEHS, Raleigh, NC.

The development of nonanimal-based testing strategies of chemicals is important in current human safety testing. Many efforts focus on the development and standardization of in vitro new approach methodologies (NAMs) that provide concentration-response data. However, concentration-response data obtained from in vitro models are inadequate for human risk and safety assessment. In order to use these data for risk assessment purposes, the in vitro concentration-response data should be translated to in vivo dose-response data. However, concentration-response data obtained from in vitro models are inadequate for human risk and safety assessment. In order to use these data for risk assessment purposes, the in vitro concentration-response data should be translated to in vivo dose-response data. However, concentration-response data obtained from in vitro models are inadequate for human risk and safety assessment. In order to use these data for risk assessment purposes, the in vitro concentration-response data should be translated to in vivo dose-response data. However, concentration-response data obtained from in vitro models are inadequate for human risk and safety assessment. In order to use these data for risk assessment purposes, the in vitro concentration-response data should be translated to in vivo dose-response data. However, concentration-response data obtained from in vitro models are inadequate for human risk and safety assessment. In order to use these data for risk assessment purposes, the in vitro concentration-response data should be translated to in vivo dose-response data.
1018 IVIVE Tools for Risk Assessment
N. Kleinsteuber, NIHES/NICEATM, Research Triangle Park, NC.
For regulators to comfortably use in vitro data and other new approach meth-
odologies in risk assessment requires translation of activity concentrations to estimated doses, which can then be compared to traditional animal toxicology studies or human exposures. The Integrated Chemical Environment (ICE; https://ice.ntp.niehs.nih.gov/) houses high-quality, curated data from the National Toxicology Program Interagency Center on Alternative Toxicological Methods (NICEATM) and its partners. The ICE Integrator currently provides access to in vitro and in vivo data for about 10,000 chemicals; endpoints include acute oral toxicity, skin irritation, eye irritation, skin sensitization, and endocrine activity, and curated high-throughput screening data from the Tox21 program. The ICE interactive workflow section allows users to select in vitro data, apply IVIVE, and compare the estimated doses to in vivo data. IVIVE models are automatically parameterized using experimental values for plasma protein binding and intrinsic clearance, where available, and in all other cases these values are predicted using high-performing quantitative structure activity relationship models. Scientific confidence can be established by quantitatively comparing estimated doses derived from validated in vitro models to regulatory guideline in vivo studies, across sets of reference chemicals. This publicly available, easy-to-use tool facilitates implementation of IVIVE in risk assessment and regulatory decision making.

1019 PBK Modeling-Based IVIVE for the Toxicological Assessment of Potential Endocrine Disruptors
E. Fabian, BASF SE, Ludwigshafen, Germany.
In order to obtain effect- or no-effect levels from non-animal methods, effect concentrations in vitro are translated to external doses, by reverse dosimetry calculations. For this approach, we extrapolated for several compounds assumed to cause a hormonal-imbalance, the lowest effect concentrations (LOEC) from in vitro assays to lowest observed effect levels (LOEL). For modeling, we applied a one compartment model and an eight compartment PBTK model. Substance specific input parameters for were the molecular weight, the plasma protein binding (PPB) and the hepatic clearance and for PBTK modeling logP and apparent permeability through Caco-2 cells (Papp) in addition. Calculations were performed for ten compounds (Bisphenol A (BPA), Fenamimot (FBPA), 17ß-Ethynylestradiol (EE), Aetaziminospongen (APAP), Caffeine (CAF), Ketocoazolone (KET), Fluamide (FLU), Genistein (GEN), Methyltestosterone (MTT) and Trenbolone (TRE)), using the lowest effect concentrations from in vitro assays addressing interaction of the test substances with estrogen- and androgen-receptors as well as steroidogenesis. The oral LOECs which were extrapolated from in vitro data were compared to LOELs obtained from in vivo studies. For the one compartment model seven and for the PBTK model six out of the 10 substances, the extrapolated and measured LOEL were in the same order of magnitude (assessed as correctly predicted). Correct predictions in both models were obtained for BPA, FEN, APAP, CAF and KET. Although less complex, the one 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, MTT, TRE). In conclusion, reverse dosimetry calculations can provide correct predictions for some substances, whereas it fails for others. There is a need to improve the models or identify substance groups for which the reverse dosimetry models are not sufficient beforehand. Increasing the number of compartments in the model is obviously not a comprehensive solution, optimizing the input parameters appears more promising.

1020 Development of a Generic Physiologically Based Kinetic Model to Predict In Vivo Endocrine Activity in Rats Based on In Vitro Bioassays
M. Zhang, Wageningen University, Wageningen, Netherlands. Sponsor: B. van Ravenzwaay
Given that the elaboration of a PBK model for individual compounds can be resource and time consuming, efforts are directed to the development of efficient and general models for large groups of compounds. The potential of a generic PBK model to predict the in vivo endocrine activities in rats for a larger series of compounds is assessed. Generic “Berkeley-Madonna” PBK models were used for the in vitro concentration-response data for estrogenic and androgenic activity from the MCF-7/BBOS proliferation assay and the yeast estrogen/androgen screening (YES/YAS) assay and translated into in vivo dose-response data. Benchmark dose (BMD) values derived from the predicted dose-response data were compared with the BMD values obtained from the in vivo Uterotrophic assay or in vivo Hershberger assay to evaluate the model predictions. Overall, the feasibility of using a combination of in vitro toxicity data and a generic PBK model to predict in vivo endocrine activities for groups of endocrine disruptors was shown. However, there were also some cases with large differences. The major source of these differences seems to be related to the variability of results in the in vitro assays.

1021 Alpha-Synuclein: A Good Protein Turned Bad in Chronic Brain Diseases with Toxicological Implications
W. Zheng, Purdue University, West Lafayette, IN.
Alpha-Synuclein (aSyn) is a low molecular weight (14.5 kDa), natively un-
folded protein expressed in a wide range of cell types and is particularly abundant in synaptic terminals. The exact function of aSyn remains un-
certain; however, recent evidence suggests that aSyn functions in the brain to maintain synaptic plasticity, regulate dopamine synthesis, and facilitate vesicular dynamics (e.g., stabilization and exocytotic fusion at presynapses). Dysfunction and aggregation of aSyn has been associated with the pathologi-
cology of Parkinson’s disease (PD), Alzheimer’s disease (AD), and Diffused Lewy Body disease (DLB). The monomeric form of aSyn in the brain mainly or-
mates from neuronal cells, yet aSyn present in the circulatory system can pass across the blood-brain barrier (BBB) to enter the brain parenchyma. Recent studies have explored the possibility of using total plasma levels of aSyn as a surrogate marker for the progression of PD and other neurodegenerative diseases such as AD. Thus, it is imperative to understand the mechanism by which the BBB regulates the fluxes of aSyn in and out of the brain to maintain aSyn homeostasis in the central milieu. Excessive aSyn proteins in brain tend to misfold, leading to massive aggregation in Lewy bodies. This aggregation is believed to be promoted by the binding of the protein to phospholipid membranes and by post-translational modifications resulting from mitochon-
drial dysfunction/oxidative stress. aSyn has divalent metal binding sites that are known to affect the protein stability. Toxicological findings support a role for aSyn in chemically induced Parkinsonian disorders; for example, exposure to beta caroline derivatives in food and manganese in the environment greatly increases aSyn aggregation. Also, certain pesticides are known to up-
regulate aSyn expression. These discoveries have established causal relation-
ships between the environmental exposure to toxic substances and altered aSyn gene expression, increased influx to brain, decreased clearance from brain parenchyma, and ultimately accelerated aSyn aggregation. This session brings together in one place the worldwide experts who are actively inves-
tigating aSyn biology, chemistry, neurotoxicity, its underlying cellular and molecular mechanisms, and clinical consequences, to address an interesting question: How does a “good” aSyn protein change to be a culprit in envi-
ronmentally linked neurodegenerative diseases? After a brief introduction of aSyn in health and human diseases, the first presenter will highlight the current understanding of mechanisms of aSyn self-assembly and how exposure to environmental toxicants promotes the protein’s aggregation. The second presenter will discuss the potential of using total and phosphorylated aSyn in the cerebrospinal fluid (CSF) and plasma to diagnose AD and PD, based on human longitudinal studies, and how this approach may be applied to neu-
rotoxicological investigations. The third presenter will extend the subject to illustrate the processes that regulate aSyn transport by the BBB and how the altered aSyn transport at brain barriers may lead to Parkinsonian disorders. The last presenter will review the latest findings showing that Mn exposure enhances the release of misfolded aSyn via exosomes by impairing endoso-
mal trafficking machinery. A sensitive high-throughput method to quantify aSyn in wielder’s serum will also be introduced. This session will present the latest discoveries on the structural, genetic, cellular, and molecular mecha-
nisms of aSyn neurodegenerative diseases, as well as a broad interest from those engaged in toxicological research of neurodevelopment and neurodegenerative diseases, neuroscience, neurotoxicology, metal toxicology, and nanoscience.

1022 How aSyn Converts from Good to Bad: A Role for Environmental Toxicants
J. Rochet, Purdue University, West Lafayette, IN. Sponsor: W. Zheng
This talk will highlight recent findings on the role of aSyn aggregation and aSyn-toxicant interactions in PD. The first part of the presentation will pro-
vide an overview of the protein’s normal physiological function and how this function is modulated by aSyn-membrane interactions. The next part of the talk will be based on mouse models of aSyn aggregation, with an empha-
sis on recent data highlighting the importance of membrane-induced aSyn aggregation in PD-related neurotoxicity. The presentation will also outline new findings suggesting a link between exposure to environmental toxicants, including pro-oxidants in pesticides or herbicides and heterocyclic amines in
Assessing Acute Health Risk: Potential Roles of Peripheral aSyn in Exposure Assessment and Risk Management


The concentration of peripheral aSyn, e.g., aSyn contained in red blood cells (RBCs), is much higher than that in the central nervous system (CNS); however, physiological and pathological roles of this pool of peripheral aSyn are essentially unknown. Recently, we have demonstrated that RBC aSyn likely contributes to CNS pathology seen in Parkinson’s disease and Alzheimer’s disease in addition to being potential biomarkers of neurodegenerative disorders. Peripheral aSyn is also an excellent candidate for interaction with neurotoxicants, especially manganese that has been shown to facilitate aSyn aggregation. Indeed, a peripheral contribution of aSyn to Parkinson’s disease and related disorders substantially strengthens the argument of environmental factors, metals in particular, involved in Parkinson’s pathogenesis. This talk will summarize recent advances in this area and potential future developments.

1024 Transport of aSyn by Brain Barrier Systems and Relevance to aSyn Toxicity


Understanding of how aSyn is transported in and out of brain is critical to predict environmental causes of aSyn-associated neurologic disorders and to design drugs for treatment. Current knowledge to this respect is surprisingly incomplete. This presentation will discuss the new finding of the cerebrovascular targets at the blood-brain barrier (BBB) that affect aSyn clearance from the brain in PD. By modifying transporters, tight junction and receptor expression using in vitro and ex vivo models of isolated brain capillary endothelial cells and SD-Tg(SNCA*A53T) transgenic rats, we have discovered that both native and mutant aSyn isoforms induce a biphasic regulation of ABC-transporters in BBB and that low aSyn concentrations cause a transient reduction of LRPI expression which turns into an up-regulation of RAGE at higher aSyn concentrations after long-term exposure. This process is accompanied by an initial tightening of the BBB but followed by a gradual opening and can be correlated to increasing levels of secreted inflammatory and oxidative stress markers. Inhibitors of clathrin-mediated endocytosis decrease the aSyn passage from the brain to the blood compartment and vice versa, implicating a receptor-mediated mechanism (e.g., RAGE, LRPI). Pathophysiological alterations in the uptake and removal mechanisms of aSyn monomers, oligomers and fibrils from either the blood or brain compartment appear to determine the extent to which toxic aSyn amasses. The presentation will further discuss the vulnerability of these cerebrovascular target sites that are subjected to insults by pesticides and metals as they disturb the BBB transport of aSyn leading to harmful aSyn accumulation in PD.

1025 Translational Relevance of Misfolded aSyn Release via Exosomes in Manganese-Induced Parkinsonian Disorder

A. Kanthasamy. Iowa State University, Ames, IA.

Emerging evidence indicates that a prion-like, cell-to-cell transfer of misfolded proteins contributes to the spreading of aSyn aggregates and neurotoxicity; but the cellular mechanisms underlying the propagation of the protein aggregates during environmental neurotoxic metal exposure are not well understood. This presentation will demonstrate that misfolded aSyn is secreted through exosomes and transfers cell-to-cell following exposures to environmentally relevant doses of manganese (Mn) in neuronal cells. Functionally, these exosomes stimulate microglial cells to activate a sustained neuroinflammatory response subsequently contributing to neurotoxicity. The data will further show that these exosomes can initiate Parkinsonian-like pathological features, including aSyn aggregation and propagation, in an animal model. The presentation will also highlight the translational relevance of our findings to the detection of aSyn aggregates using an ultra-sensitive high-throughput RT-QuIC diagnostic assay in the serum exosomes of welders occupationally exposed to manganese. Overall, this presentation will offer a comprehensive insight into pathological mechanism mediated by aSyn aggregation in chemical induced Parkinsonism as well as potential biomarker value of the protein aggregates in environmentally linked neurodegenerative diseases.

1026 Assessing Acute Health Risk: Potential Application of Next-Generation Toxicological Tools

M. Stewart. US EPA, Research Triangle Park, NC.

Human health risk assessments estimate the nature and probability of adverse health effects in humans who may be exposed to chemicals in the environment. Estimates of health risks resulting from chemical exposure are typically based on an assumption that either low-level, chronic (=year or more) or higher-level, acute (=day or less) exposures adequately span the range of all potential scenarios. However, there are relatively few health effect toxicity values applicable to the general population for acute exposures as opposed to chronic chemical exposures. A primary reason is that a large number of chemicals have a paucity of short-term human and animal data on which to base acute toxicity values. In vitro, computational modeling, conceptual frameworks, and other higher-throughput measurements may help fill these gaps. These next-generation tools have been slowly integrated into chronic chemical hazard prioritization over the last decade, but the use of these tools to quantitatively derive reference values for acute exposure scenarios has not been widely discussed even though these studies are inherently acute in nature. This session will first explore how acute toxicity values are currently being derived and used to assess the potential for short-term risk. The session will continue to discuss both the challenges and the promises of using next-generation toxicological tools (specifically pharmacokinetic or pharmacodynamic modeling, adverse outcome pathway frameworks, and high-throughput testing strategies) to address data deficiencies associated with the derivation and proper application of acute health toxicity values.

1027 Exposure Assessment and Risk Management Using Acute Toxicity Factors to Evaluate Ambient Air Monitoring Data in Texas

T. Bredfeldt. Texas Commission on Environmental Quality, Austin, TX.

In response to the need for exposure assessment of ambient air pollutants, the Texas Commission on Environmental Quality (TCEQ) has designed the largest ambient air monitoring network in the U.S. The agency lists a total of 256 fixed-site monitors in the state, which are owned by various entities including the TCEQ. The Toxicology Division evaluates data from 129 of those sites, which collect concentrations of metals, volatile organic compounds, carbonyls, and polycyclic aromatic hydrocarbons. Concentrations are reported as 30-minute, 1-hour, 3-hour, 24-hour, or annual average concentrations. In order to evaluate data collected at these monitoring sites, the Toxicology Division staff derive chemical-specific toxicity factors that are used as comparison values when assessing chemical concentrations measured in ambient air throughout the state. Given the extensive nature of the air monitoring network in the state, the TCEQ is able to evaluate whether chemical concentrations observed in various locations would be of concern to human health or welfare. Further, the large amount of short-term air data collected enables the agency to have a unique ability to evaluate patterns and trends in short-term ambient air samples, including 30-minute and 1-hour peaks, the frequency of toxicity factor exceedance, region-specific chemicals of concern, and chemicals measured in specific environments such as the Barnett Shale play or the heavily industrialized Houston and Corpus Christi ship channels. This presentation will describe the methods used by the TCEQ to derive chemical-specific toxicity factors. Then, using a number of monitoring sites as case studies, we will present data describing trends in air monitoring data as a function of time, the frequency of toxicity factor exceedances, potential sources, and how these compare to monitoring in rural or suburban areas populated with fewer sources. These data are useful in characterizing and understanding potential health risks associated with acute exposure to various chemicals measured in the environment. Such data are critical to problem formulation and risk management of acute and chronic chemical exposures and could be used as a model to further inform risk assessors and managers in other states about chemical-specific, risk management strategies.
A Conceptual Model for Predicting How Acutely Toxic Exposure Levels Should Relate to Those Associated with Toxicity from Longer-Term Exposures, Suggesting Approaches to Using In Vitro Data in Exposure-Duration Extrapolation

L. Rhomberg, Gradient, Cambridge, MA.

The relationship between exposure levels causing acute toxicity and those (usually lower) that are associated with repeat-dose toxicity hinges on the impact of dose rate. Dose-rate effects, in turn, can depend mainly on underlying pharmacokinetics (PK) or pharmacodynamics (PD). Earlier work examined how equally toxic combinations of acute inhalation exposure level and exposure duration can be understood in terms of the balance between uptake and clearance (for PK) and of damage and repair (for PD), with the process taking longest to reach steady state driving the overall pattern. This now extends the analysis to oral exposures and to longer-term exposures, providing a conceptual framework to predict how dose-rate effects should intervene to result in different acutely toxic dose levels compared to those having effects after ongoing or repeat exposure. This includes brief consideration of how adverse outcome pathways (AOPs) might be examined to identify events that are likely to be drivers of dose-rate effects, and how in vitro evaluation of damage and repair processes related to such key events might be useful in predicting sensitive endpoints for acute toxicity as well as the relationships between acutely toxic exposures and ones associated with longer-term toxicity.

Application of Modern Toxicology Approaches for Predicting Acute Toxicity

D. Dorman, North Carolina State University, Raleigh, NC.

There is a critical need to develop modern approaches for predicting acute, debilitating chemical toxicity. This presentation will present major conclusions from a recent National Academies report entitled Application of Modern Toxicology Approaches for Predicting Acute Toxicity for Chemical Defense. This presentation will discuss the current state of computational and high-throughput approaches for predicting acute toxicity. The National Academies multi-tiered prioritization strategy that consists of initial character- ization, nontesting approaches (e.g., quantitative structure–activity relationship [QSAR] models), high-throughput and medium-throughput assays, and ultimately traditional animal testing will also be discussed. This strategy rests on the hypothesis that chemical structure, physicochemical properties, biochemical properties, or biological activity in isolated cells and tissues or in nonmammalian organisms can be used to predict acute mammalian toxicity. Strengths and limitations of many in vitro assays for predicting acute toxicity were identified by the National Academies and will also be discussed. For example, quantitative linkage of the endpoints used (e.g., cytotoxicity) to a phenotype is often lacking, assays do not account for important pharmacoki- netic characteristics that can influence in vivo toxicity, and nominal chemical concentration used in the assays may not reflect concentrations found in vivo.

Predicting Chemical Affinity and Extrapolating to Safe Acute Exposure Levels (SAELs)

L. Burgoon, US Army Engineer Research and Development Center, Vicksburg, MS.

Warfighters and first responders are at significant risk of acute exposure to chemicals following terrorist attacks, or as the result of traps being triggered in the course of duty. Several US Federal Agencies and stakeholders had been developing Acute Exposure Guidelines (AEGs) for chemicals in the past; however, that program has recently ceased due to a lapse in funding. Thus, there are still thousands of potentially acutely toxic chemicals that lack an assessment. This talk will discuss our strategy to predict safe acute exposure levels (SAELs) using chemical structures as a starting point. The SAELs approach will be demonstrated using the artificial intelligence chemical affinity models that we have built to extrapolate from chemical structures to protein binding affinity and protein activation for the mu-opioid receptor and acetylcholines- terase. We will see how we use this information to inform us about potential adverse outcomes by looking at Adverse Outcome Pathway Networks available in AOPConnect. We will also discuss our approaches for generating chemical binding information to fill in data gaps using the AEGs or other available information. The US Army Chief of Engineers has approved this information for release. The views presented in this article do not necessarily reflect current or future opinion or policy of the US Army Corps of Engineers.

Session Overview: Bringing Next-Generation Toxicological Tools into Acute Health Risk Assessments

G. Woodall, US EPA, Research Triangle Park, NC.

The purpose of this presentation is to provide a holistic overview of the prior presentations and to predict how the next generation toxicological tools can be applied to improve acute human health risk estimates. Traditionally, human health risk assessment from chemical exposures rests on the ability to either observe toxicity to humans directly, or to predict toxicity based on observations in laboratory animals exposed to the chemical of interest. If data for the chemical are not available, estimates may be based on “read-across” from observations with chemicals anticipated to have sufficiently similar structure and biological activity. Modeling approaches have been used to provide more precise predictions using known biological parameters. A more complete understanding of the key events and mechanisms are being eluci- dated at a more granular level by the next generation of high-throughput and in vitro testing platforms, leading to improved predictions through construct- ing adverse outcome pathways (AOPs). Many of these topics are covered to some extent in prior presentations in this session; this talk will discuss how the more recently developed approaches to predicting toxicity might realistically become incorporated into risk assessments - particularly for acute exposures. Discussion will touch upon the current methods used to establish acceptable acute exposures, examine and challenge some of the assumptions used in traditional acute risk predictions, and discuss how more recently developed data streams and analytical approaches may be used to either improve or fill-in data gaps for establishing risk estimate values for acute exposures. The views in this abstract are those of the author and do not necessarily represent the views or policy of the US EPA.

Novel Genetic-Based Tools for Evaluating Toxicity Potential, Mechanism of Action, and Population Dynamics

B. Cummings, University of Georgia, Athens, GA.

The genetic information encoded in DNA and perturbations in the flow of genetic information from DNA to downstream biomolecules (i.e., RNA then protein) following chemical exposure are critical determinants of toxicologi- cal responses in terms of both functional outcomes and variations in response across a population (i.e., inter-individual susceptibility). In general, toxicolo- gists have been cautious regarding when and how to incorporate information about genetically controlled responses or genetic susceptibility into safety and risk assessment pipelines. However, there are a number of recently de- veloped molecular biology tools that can be used to evaluate the role of ge- netic structure, gene regulation, and gene expression in response to drug and chemical exposures that provide informative data for use in various steps across risk evaluation pipelines. The aim of this session is to discuss emerg- ing systems genetics tools that may help advance toxicological evaluation and to present case studies demonstrating their utility. Tools and approaches that will be discussed in this session include (1) the use of genetically diverse population models for investigating the contribution of genetic sequence variation to toxicant susceptibility and applications toward replacement of default toxicodynamic uncertainty factors, (2) the use of gene-editing technol- ogies in a screening context toward discovery of key genetic drivers under- lying susceptibility and resistance to toxicant exposures, (3) the use of methylome-based next-generation sequencing (NGS) for rapid assessment of toxicant-induced changes in DNA methylation patterns and applications for adverse outcome pathway (AOP) integration, (4) the use of targeted RNA-seq in a high-throughput transcriptomics (HTTR) screening context and methods for in vitro point-of-departure estimation and in vitro to in vivo extrapolation (IVIVE), and (5) the use of weighted gene co-expression networks (WGCNA) for toxicant mode-of-action analysis. Participants in the session will gain a broader understanding of emerging genetic and transcriptomic analysis tools, their strengths and limitations, and their applications in testing priori- tization, mechanistic analysis, and human health risk assessment.

Quantifying Inter-Individual Toxicodynamic Variability Using Genetic Reference Populations to Inform Risk Assessment

A. Harrill, NIEHS/NTP, Research Triangle Park, NC.

Toxicology testing is often performed in very limited genetic context (human cells, rodent strains) that do not represent the genetic diversity of human pop- ulations. The question then becomes, which humans is this cell line or rodent strain representing, and how can these results be translated to genetically diverse humans? To overcome these challenges, novel outbred rodent stocks,
such as Diversity Outbred (DO) mice, have been developed with genomes that have a highly randomized allelic architecture and for which each individual is genetically unique (like humans). DO mice can thus act as a population surrogate for human epidemiological studies, allowing investigators to query toxicodynamic variability in responses and their genetic drivers. Data will be presented in which neural progenitor cells from DO mice in vitro are used to assess the impact of toxicants on gene expression. TempO-Seq is a targeted RNA-Seq approach to perform genome-wide functional toxicogenomic analyses. This approach allows for rapid and cost-effective assessment of DNA methylation of a significant portion of the genome, but are relatively expensive. Additionally, MeDIP-Seq assesses a small amount of DNA per cell, while whole genome bisulfite sequencing (WGBS) is rapid and cost effective, but assesses a small amount of DNA per cell. The population-based in vitro data may thus provide regulators with a rationale to replace default uncertainty factors for toxicodynamic variability in setting human dose thresholds.

1034 Genome-Wide and Targeted CRISPR Functional Approaches in Toxicology


Comprehensive identification of cellular pathways involved in chemical toxicity response has recently become more feasible due to the emergence of the CRISPR clustered regularly interspaced short palindromic repeats (CRISPR) genome editing technique. This allows for genome-wide functional toxicogenomic approaches. We used genome-wide CRISPR knock-out screening to identify genes whose loss-of-function alters sensitivity to acetaldehyde and arsenic trioxide. Our screens identified multiple genes that impact cellular toxicity of each of the studied chemicals. Consistent with the reported role of aldehydes in DNA damage, we demonstrated that disruption of aldehyde dehydrogenase (ALDH) gene encoding the enzyme ALDH1A1 increased the toxic effect of acetaldehyde. In addition, we identified a gene of unknown function, OVC2, which modulates acetaldehyde adduct levels in DNA. Confirmatory studies demonstrated that CRISPR-mediated ablation of this gene increased the levels of acetaldehyde-DNA adducts. We hypothesize a role for OVC2 in adduct removal although we cannot rule out a direct role in acetaldehyde metabolism. Our study on arsenic trioxide identified multiple cellular components involved in mitigating the effects of reactive oxygen species (ROS) as playing important roles in acute ATO toxicity. We also revealed a novel cellular link between arsenic toxicity and selenocysteine metabolism. We found that disruption of any of the multiple genes involved in selenocysteine biosynthesis increased arsenical resistance and we discuss two possible hypotheses to explain these findings. Further, we identified key cellular components involved in arsenical uptake and export. Our work further demonstrates the strength of high throughput screening using the CRISPR-Cas9 system in deciphering mechanisms of toxicity.

1035 Novel Methods for Rapid Assessment of Toxicant-Induced Changes in DNA Methylation

B. Cummings. University of Georgia, Athens, GA.

Technologies are presented for assessment of changes in DNA methylation in response to toxicant exposure. Some of these approaches, such as pyrosequencing, are rapid and cost effective, but assess a small amount of DNA per sample. Others, such as methylated DNA immunoprecipitation sequencing (MeDIP-Seq) and the Infinium MethylationEPIC BeadChip®, assess a significant amount of the genome, but are relatively expensive. Additionally, MeDIP-Seq offers more qualitative regional methylation analysis with less resolution, as opposed to quantitative assessment of single differentially methylated sites. While the Infinium MethylationEPIC BeadChip® assesses changes at single sites, it is not as cost effective as MeDIP-Seq, and both techniques offer less dynamic range than reduced representation bisulfite sequencing (RRBS). Most of these techniques are typically used for whole genome analysis. We present case studies highlighting comparisons of methylation analysis platforms including MeDIP-Seq, Illumina 450K and EPIC Beadchip®. We also discuss key factors in variability in DNA methylation analysis including bisulfite conversion and library preparation. We present data derived from a modification of RRBS called targeted bisulfite next generation sequencing (TB-NGS) that allows for rapid and cost-effective assessment of DNA methylation of single gene targets. TB-NGS was used to determine changes in the methylation of the cyclin-dependent kinase inhibitor p21 in response to bromate (a single gene targets. TB-NGS was used to determine changes in DNA methylation of the promoter region of human p21 whose methylation was mediated by DNA-methyl transferase POL3 and the novel CRISPR-Cas9 system in Vtg whose methylation was decreased 50% after EE2 exposure.

1036 High-Throughput Transcriptomics (HTTr) Screening with Targeted RNA-Seq: Applications for In Vitro Point-of-Departure Estimation and In Vitro to In Vivo Extrapolation

J. Harrill. US EPA, Research Triangle Park, NC.

Current initiatives in the field of environmental toxicology include integration of toxicity testing data from in vitro and alternative methods into risk assessment practices. Approaches have been proposed that use potency estimates of bioactivity from in vitro testing alone, coupled with high-throughput toxicokinetic predictions, to calculate human equivalent doses that can, in turn, be used as the basis for screening-level human health risk assessment. The increasing efficiency and declining cost of generating whole transcriptome profiles has made high-throughput transcriptomics (HTTr) a practical option for determining bioactivity thresholds in in vitro models. Recently, US EPA NCTs have performed concentration-response screening of 2,200 chemicals from the ToxCast chemical inventory in MCF-7 cells using the TempO-Seq human whole transcriptome targeted RNA-Seq assay. The TempO-Seq technology has also been used to investigate the effects of volatile chemicals such as acrolein and 1,3-butadiene on gene expression in BEAS-2B and human primary bronchial epithelial cells grown in an air-liquid interface. This presentation will provide a broad overview of the results of these studies and particularly focus on methods and tools for high-throughput concentration-response modeling and in vitro POD estimation in each model. Margin of exposure (MOE) case studies will be presented where the human equivalent doses calculated from HTTr data compare well to bioactivity thresholds that will be compared to those based on other high-throughput in vitro data streams (i.e. ToxCast assays) as well as those generated via high-throughput exposure prediction. HTTr-derived, biological pathway activating concentrations (BPACs) for promiscuous ToxCast chemicals (i.e. those active in dozens to hundreds of ToxCast assays) were often of an order of magnitude lower than the corresponding ToxCast PODs, potentially providing greater resolution and sensitivity for detecting perturbation of sensitive biological processes. This abstract does not necessarily reflect US EPA policy.

1037 TXG-Map: Systems Biology Approaches to Understanding Adverse Outcomes

Y. Webster. Eli Lilly and Company, Indianapolis, IN. Sponsor: J. Harrill

Despite investment in toxicogenomics, nonclinical safety studies are still the mainstay to predict clinical liabilities for new drug candidates. Network-based approaches for genomic analysis help overcome challenges with whole-genome transcriptional profiling using limited numbers of treatments for phenotypes of interest. In this talk, a co-expression network analysis platform, TXG-Map, will be described and applied to safety assessment using rat liver gene expression data. To develop the TXG-Map, we utilized data from two large publicly available gene expression repositories, Drug Matrix (DM) and the open TG-GATEs (TG). Using weighted gene co-expression network analysis (WGCNA) and 9071 liver-expressed genes, we defined 415 co-expressed gene modules. Annotation of the TXG-Map is provided based on pathway and Gene Ontology (GO) term enrichment as well as literature curation at a level of granularity sufficient to identify biological processes associated with co-expression networks. Changes in specific networks are interpreted in the context of changes in the entire set of networks to produce a provisional mode of action hypothesis. Some of these unique modules were highly correlated with liver toxicity phenotypes. For example, tunicamycin is a prototypical ER stress inducer. In one case study, we tried to identify other compounds that induce ER stress and caused similar pathology. We searched all module profiles (i.e. describing each as a vector of 415 module scores) using tunicamycin as a query. The most similar compound was ethanomide, a second line therapy for mycobacterial infection, with hepatotoxicity liability. Further analyses showed that among modules most induced by both chemicals, module 70 contains Ddit3 and was top-ranked for single-cell necrosis/apoptosis and module 76 contains canonical ER chaperones Hspa5 and Hsp0901b (Grp94). Notably, module 85 was also induced by both compounds and contains Atf4 and Atf4-target genes. When we treated rat primary hepatocytes with ethaminothiurea, there was a concentration-dependent increase in Atf4, Grp78 and...
Endocrine disruption is a major health concern for many persistent environmental chemicals, with adverse outcomes in metabolism, development, reproduction, and cancer. The endocrine systems are robust nonlinear dynamical systems by nature, which can resist perturbations to maintain hormone homeostasis through feedback regulations between multiple organs. Simple linear extrapolation from high-dose data to environmental low-dose effects is thus not applicable to predicting the health risk of endocrine-disrupting chemicals (EDCs). Moreover, as toxicity testing is increasingly shifting to cellular or organoid-based in vitro assays, the demand for in vivo extrapolation that can predict systems-level hormonal outcomes and apical endpoint consequences in human populations is also rising. Bridging these data gaps calls for a computational systems biology approach to mechanistically model the endocrine systems and their responses to perturbations by endocrine disruptors acting via diverse molecular initiating events (MIEs). This session is organized to present the state-of-the-science in mathematical modeling of endocrine systems in the context of chemical risk assessment through presentation of a series of computational works on thyroid, reproductive, and adrenal systems. The first presenter lays out the general design principles for the homeostatic regulation of the endocrine systems involving feedback interactions between the hypothalamus, pituitary, and endocrine organs. Using the thyroid system as an example, the presentation illustrates how mechanistic computational models constructed according to these principles and incorporating individual variability can aid the interpretation and quantitative prediction of the health outcomes of EDCs. The second talk presents a more detailed model of the thyroid system to understand the effect of iodine nutritional status on thyroid hormone levels during pregnancy and lactation. While the model is unable to explain why repletion of iodine intake cannot ameliorate the effect of iodine deficiency, it provides mechanistic insights into the dynamic functioning of the hypothalamic-pituitary-thyroid (HPT) axis during the reproductive stage of women. The third talk presents the modeling work on in vitro to in vivo extrapolation (IVIVE) for the risk effects of EDCs on both the hypothalamic-pituitary-adrenal axis and male reproductive systems. By using in vitro data from thyroperoxidase and sodium-iodide symporter inhibition assays and a purified rat Leydig cells assay that detects alterations in testosterone production, the speaker illustrates quantitative adverse outcome pathway (qAOP) models that can extrapolate in vitro data to predict mammalian neurodevelopmental deficits. The fourth talk presents the models on the hypothalamic-pituitary-adrenal axis and how its interaction with the circadian rhythm can affect between-sex and within-sex variability. The model provides a computational tool that can be tapped to understand the heterogeneous responses in human populations to stress and to aid risk assessment of EDCs interfering with the physiological stress response. The final presentation includes a comprehensive modeling framework that links exposure, toxicokinetic, and ovarian cycle models. The work illustrates how modeling the exposure-to-outcome continuum through linking these models and incorporating ToxCast assay data can aid in the identification of putative inhibitors on menstrual cycle length and ovulation. In summary, the session will demonstrate that through integrating chemical and biological data from in vitro, in vivo, epitoxinology, and exposure studies, computational systems biology models of endocrine systems can play a key, bridging role in quantitatively understanding and predicting the health risks of EDCs.

**1038 Application of Computational Modeling to Risk Assessment of Endocrine Disruptors**

Q. Zhang, Emory University, Atlanta, GA.

Indicates that the biochemical amplifier, required to achieve a high feedback loop gain, is located in the brain not in the thyroid gland. Multiple signaling events in the hypothalamus and anterior pituitary collectively contribute to a highly ultrasensitive motif that amplifies small changes in TH levels to regulate TSH synthesis and secretion. This design minimizes the chance of thyroid hormone alteration by protecting the sensitive signaling part within the blood-brain barrier. Depending on the molecular initiating event (MIE), EDCs affecting TH synthesis, metabolism, and action can exhibit different shape of dose-response curves for their effects on the hormone levels in the low-dose region. The inverse relationship between TSH and THs observed in the human population suggests that most sources of individual variabilities stem from the peripheral tissues whereas the pituitary system is relatively consolidated. Although this screening can be useful in examining the potential of chemicals to disrupt the hypothalamic-pituitary-thyroid axis, it provides mechanistic insights into the dynamic functioning of the hypothalamic-pituitary-thyroid (HPT) axis during the reproductive stage of women. The third talk presents the modeling work on in vitro to in vivo extrapolation (IVIVE) for the risk effects of EDCs on both the hypothalamic-pituitary-adrenal axis and male reproductive systems. By using in vitro data from thyroperoxidase and sodium-iodide symporter inhibition assays and a purified rat Leydig cells assay that detects alterations in testosterone production, the speaker illustrates quantitative adverse outcome pathway (qAOP) models that can extrapolate in vitro data to predict mammalian neurodevelopmental deficits. The fourth talk presents the models on the hypothalamic-pituitary-adrenal axis and how its interaction with the circadian rhythm can affect between-sex and within-sex variability. The model provides a computational tool that can be tapped to understand the heterogeneous responses in human populations to stress and to aid risk assessment of EDCs interfering with the physiological stress response. The final presentation includes a comprehensive modeling framework that links exposure, toxicokinetic, and ovarian cycle models. The work illustrates how modeling the exposure-to-outcome continuum through linking these models and incorporating ToxCast assay data can aid in the identification of putative inhibitors on menstrual cycle length and ovulation. In summary, the session will demonstrate that through integrating chemical and biological data from in vitro, in vivo, epitoxinology, and exposure studies, computational systems biology models of endocrine systems can play a key, bridging role in quantitatively understanding and predicting the health risks of EDCs.

**1039 Design Principles of Endocrine Systems and Their Applications to Understanding Endocrine Disruptions: A Case Study with the Hypothalamic-Pituitary-Thyroid Axis**

Q. Zhang, and Z. Shi. Emory University, Atlanta, GA.

The endocrine systems are one of the most robust physiological structures in animals and humans. Understanding nature’s design principles for biological robustness that maintains hormone homeostasis through mechanistic computational modeling will aid the interpretation and quantitative prediction of the health outcomes of endocrine disrupting chemicals (EDCs). In this presentation, using the hypothalamic-pituitary-thyroid (HPT) axis as an example, we illustrate how the key design features help understand the thyroid effects of EDCs quantitatively. The circulating thyroid hormones (THs) are maintained in a narrow range (< 2 fold), whereas thyroid-stimulating hormone (TSH) can vary much more widely (> 10 fold). This contrasting variability profile indicates that the biochemical amplifier, required to achieve a high feedback loop gain, is located in the brain not in the thyroid gland. Multiple signaling events in the hypothalamus and anterior pituitary collectively contribute to a highly ultrasensitive motif that amplifies small changes in TH levels to regulate TSH synthesis and secretion. This design minimizes the chance of thyroid hormone alteration by protecting the sensitive signaling part within the blood-brain barrier. Depending on the molecular initiating event (MIE), EDCs affecting TH synthesis, metabolism, and action can exhibit different shape of dose-response curves for their effects on the hormone levels in the low-dose region. The inverse relationship between TSH and THs observed in the human population suggests that most sources of individual variabilities stem from the peripheral tissues whereas the pituitary system is relatively consolidated. Although this screening can be useful in examining the potential of chemicals to disrupt the hypothalamic-pituitary-thyroid axis, it provides mechanistic insights into the dynamic functioning of the hypothalamic-pituitary-thyroid (HPT) axis during the reproductive stage of women. The third talk presents the modeling work on in vitro to in vivo extrapolation (IVIVE) for the risk effects of EDCs on both the hypothalamic-pituitary-adrenal axis and male reproductive systems. By using in vitro data from thyroperoxidase and sodium-iodide symporter inhibition assays and a purified rat Leydig cells assay that detects alterations in testosterone production, the speaker illustrates quantitative adverse outcome pathway (qAOP) models that can extrapolate in vitro data to predict mammalian neurodevelopmental deficits. The fourth talk presents the models on the hypothalamic-pituitary-adrenal axis and how its interaction with the circadian rhythm can affect between-sex and within-sex variability. The model provides a computational tool that can be tapped to understand the heterogeneous responses in human populations to stress and to aid risk assessment of EDCs interfering with the physiological stress response. The final presentation includes a comprehensive modeling framework that links exposure, toxicokinetic, and ovarian cycle models. The work illustrates how modeling the exposure-to-outcome continuum through linking these models and incorporating ToxCast assay data can aid in the identification of putative inhibitors on menstrual cycle length and ovulation. In summary, the session will demonstrate that through integrating chemical and biological data from in vitro, in vivo, epitoxinology, and exposure studies, computational systems biology models of endocrine systems can play a key, bridging role in quantitatively understanding and predicting the health risks of EDCs.

**1040 Using Computational Approaches to Understand the Hypothalamic-Pituitary-Thyroid (HPT) Axis for Iodine Sufficient and Insufficient Conditions during Lactation**


Iodine insufficiency remains a public health concern in many regions of the world. Even for developed countries questions are raised about iodine sufficiency during pregnancy and lactation because of the critical importance of iodine during this sensitive life stage. Biologically-based HPT axis models for iodine sufficiency and insufficiency in lactating rats, nursing pups, lactating women and nursing infants revealed substantial compensatory actions, perhaps mediated by more than serum TSH. Iodine intake studies were undertaken with pregnant and lactating rats ranging from insufficient to excess. For lactating women several published studies were used to create a biologically-based computational model of the HPT axis for moderate iodine insufficiency. Iodine intervention studies, for which biomarker data exist on the status of the HPT axis, remain divergent, without a mechanistic understanding for how recovery from iodine insufficiency occurs. Our model, while generally successful, was unable to explain findings where increased dietary intake of iodine failed to ameliorate human iodine deficiency as measured by serum thyroid hormones and urinary excretion of iodide. These baseline nutritional studies serve as a first step in better understanding the complex interactions of chemicals on the HPT axis. This presentation provides mechanistic insights into the dynamic functioning of the HPT axis in rats and humans during lactation and iodine deficiency. With continued improvement this computational tool will be able to interpret chemical-induced disruption of the HPT axis in rat pups relative to the risks for HPT disruption in human neonates and infants.

**1041 Biologically Based Computational Models for Endocrine Disruption Incorporating Adverse Outcome Pathways and High-Throughput Toxicity In Vitro Testing**

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An adverse outcome pathway (AOP) is a conceptual framework of biological relationships starting from modulations of molecular initiating events (MIEs) to key events (KEs) leading to pathological adverse outcomes. The process of constructing AOPs requires the accumulation of data and knowledge from experimental studies, literature and publicly available databases. The immediate application of AOPs is the screening of chemicals using information from in vitro high-throughput toxicity (HTT) experiments applied to specific MIEs. Although this screening can be useful in examining the potential of chemicals to cause adverse outcomes, it is not enough for estimating health hazards risks to humans. This inadequacy is due to differences in the behaviors and effects of chemicals in vitro vs. in vivo. For this reason, in vitro to in vivo (IVIVE) extrapolation of KEs in an AOP becomes an essential process to fully examine the hazard risks of environmental chemicals. Additionally, in the absence of chemical influence, AOPs provide the biological framework for developing computational predictive models (qAOPs) to quantitatively describe the sequence and timing of effects (key event relationships, KERs) leading to the pathogenesis of an AOP. In this manner, qAOPs are used to computationally estimate dose-response relationships where the effective dose can
be a chemical-independent biomarker (e.g. thyroid hormone levels in fetal brain) and the response is a pathological end point related to an adverse outcome (e.g. fetal brain heterotopia size). This presentation will provide details for the applications of biologically-based modeling to 1) conduct IVIVE of in vitro purified rat Leydig cell assay to predict chemical-induced alterations in testosterone production, and 2) develop qAOPs for thyperoxidease (TPO) and sodium-adrenyloxytoxin (NIS) inhibition leading to mammalian neuro-developmental deficits. The application of computational methods to predict dose-response relationships in view of in vivo biological mechanisms as outlined in an AOP construct is critical in the estimation of health risk estimates of environmental chemicals where data is not sufficient.

### W 1042 Personalized Adaptation to Stress and Physiological Trade-Offs in the Circadian Regulation of the HPA Axis: A Systems Biology Approach


Epidemiological studies established that exposure impairs the cardiovascular, respiratory and central nervous systems leading to a range of disease states. Exposure activates the hypothalamic-pituitary-adrenal (HPA) axis – a key component of the stress response – leading to increased levels of corticosterone/cortisol initiating systemic adverse effects. Evidence suggests that endocrine disruptors (pesticides, flame retardants and UV-filter chemicals) perturb circadian rhythm. The HPA exhibits complex dynamics resulting in robust circadian patterns driving the dynamics of glucocorticoid hormones. Persistent disruption of homeostatic glucocorticoid circadian rhythmicity due to chronic exposure is correlated with the incidence of a range of pathological conditions. A hallmark of HPA dynamics is its stress response disparity mediated by regulatory plasticity in its activity. Sex differences in HPA activity are prominent and thought to contribute to sex-specific disparity in the prevalence of stress disorders. We discuss mathematical models characterizing differences in regulatory properties controlling the circadian dynamics of the HPA axis contributing to sex-specific and inter-individual variability and stress-responsive functioning. We focus on understanding sex-dependent sensitivities in the dynamics of the HPA, hypothesizing that regulatory plasticity enables HPA adjustments to maintain homeostasis under stress and demonstrating that adaptation comes at a cost (allostatic load). We identify distinct sex-specific parameter spaces of greater adrenal sensitivity and weaker negative feedback in females indicative of the inter-individual variability in the HPA regulatory mechanisms. Allostatic habituation of HPA regulation provides fitness advantages by preventing the sustained dysregulation of glucocorticoid-responsive signaling pathways. Allostatic adaptation results in physiologic cost impairing the homeostatic stress-responsive and stress-responsive functioning. The HPA in the form of trade-off between the two objectives. Finally, the allostatic regulatory adaptations are predicted to cause time-of-day dependent sensitization of the acute stress response and impair the entrainability of the HPA. Our HPA model capturing individual variability provides a basis for a mechanistic tool that will allow health risk assessment of endocrine disruptors.

### W 1043 Computational Predictive Analysis of Ovarian Cycle Disruption by Mixtures of Endocrine Disruptors

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The regulation of menstrual cycle in women is controlled by coordinated hormonal stimulation and inhibition along the hypothalamic-pituitary-ovarian axis. Exposures to endocrine disruptors that interfere directly or indirectly with any of the hormones responsible for this process can induce pathological outcomes such as infertility. In this context, the role of aromatase enzyme is critical as it reversibly converts testosterone to E2, and androstenedione to estrone, maintaining the dynamic balance between androgens and estrogens. In this talk a computational approach designed to predict the effects of exposure to large-scale (i.e., potentially real-life) mixtures of aromatase inhibitors on the dynamics of menstrual cycling in women will be presented. The approach was designed over four computational steps: step one, the ExpoCast database of exposure estimates were used to simulate random exposures to potential mixtures of aromatase inhibitors. In step two, a pharmacokinetic model was developed to estimate the intake and disposition of the chemicals and predict their internal concentrations as a function of time (up to 2 yr). The robustness of the PK model was validated. Successfully in step three, the extent of inhibition of aromatase by the chemical mixture was estimated by using the concentration-inhibition relationships provided in the ToxCast database (86 chemicals). The computed results served as an input for step four, an implementation of the ovarian cycle model. Results showed that the inhibition of estradiol synthesis by aromatase inhibitor mixtures was exceedingly higher than the effects of exposure to individual chemicals (more than 10% inhibition). In fact, no effects can be expected from typical exposures to individual chemicals when considered alone. The possibility to predict large-scale mixture effects for endocrine disrupters with a predictive toxicology approach that is suitable for high-throughput ranking and risk assessment will be introduced. Lastly, the consistency of the size of the predicted effects with an increased risk of infertility in women from everyday exposures to our chemical environment will be demonstrated.

### W 1044 MALDI Tissue Imaging: A New Tool for Making TK/TD Connections to Histopathology

L. Schnackenberg. US FDA/NCTR, Jefferson, AR.

Matrix-assisted laser desorption/ionization imaging mass spectrometry, or MALDI IMS, is an emerging label-free technology, which can provide the spatial distribution of drugs, drug metabolites, lipids, and other endogenous analytes in tissue samples. The analyte tissue distributions, when correlated with histopathology, provide detail that may allow a better understanding of a drug’s mechanism of action or the effects of a toxicological insult. MALDI IMS provides high spatial resolution (10 µm), is highly sensitive, and can be quantitative, qualities that have propelled the use of MALDI IMS across a variety of disciplines, including toxicological applications. This session will explore the recent incorporation of MALDI IMS to inform TK/TD drug decisions, a tool for identifying biomarkers related to histopathology and toxicity, and a method for identifying n-linked glycans across a variety of diseases and therapeutic models. Presenters representing government, academia, and pharmaceutical sectors will share recent MALDI IMS data from toxicology studies. The first presentation will provide a brief overview of MALDI IMS, including its strengths and limitations as a new tool for toxicology. The second presenter will discuss the role of MALDI-FTICR imaging to assist the United States Department of Defense at Fort Detrick to evaluate changes in metabolites in relation to infection from viral or bacterial pathogens. The third presenter will discuss the recent incorporation of MALDI IMS to assess drug, metabolites, and histopathological changes in a zebrafish model of drug-induced kidney toxicity. The fourth presentation will present case studies in drug development whereby MALDI IMS was utilized to better understand PK/PD relationships within a drug development pipeline. Finally, a cutting-edge and recent application of MALDI IMS will be presented, whereby n-linked glycan distributions can be identified in formalin-fixed paraffin-embedded (FFPE) tissues and microarrays from cancer biopsies. Use of this approach to further understand how glycan profiles and glycoproteins respond to therapeutics or xenobiotic exposures also will be discussed.

### W 1045 MALDI IMS: An Emerging Technology in the Field of Toxicology

L. K. Schnackenberg. US FDA/NCTR, Jefferson, AR.

Matrix assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS) is a label free, robust and emerging technology which produces 2D ion density maps representing the distribution of an analyte across a tissue section in relation to tissue histopathology. Although MALDI IMS was initially developed to spatially profile proteins and peptides, the variety of analytes which are currently being evaluated have greatly increased and include: small molecule drugs and metabolites, lipids and glycans. Incorporation of high resolution Fourier transform ion cyclotron resonance (FTICR) MALDI mass spectrometry instruments within imaging workflows has also allowed for the detection of unique and lower abundant classes of analytes such as neurotransmitters to allow their placement into metabolomic pathways of interest. Although MALDI IMS has been used for some time to study different disease models, it has recently become an attractive technology in drug toxicity studies to complement standard quantitative techniques. While the technology has limitations including the ability to accurately quantify analytes of interest the value of MALDI IMS lies in the ability to provide a spatial component to a drug or metabolite rather than an average concentration within homogenized tissue; it is an analytical technique which directly links analyte distributions to histopathology and can provide functional detail can be obtained in relation to the distribution of an analyte highlighting the power of MALDI IMS in assessing toxicology.
MALDI-FTICR Mass Spectrometry Imaging Reveals Dysregulated Lipid and Small Molecule Metabolites in Tissues Harvested from Mice Infected with a Bacterial or Viral Pathogen

L. H. Cazares. US Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD. Sponsor: L. Schnackenberg

Few studies have examined in-depth, the metabolic dysregulation and cross-talk which occurs between a pathogenic bacterium or virus and a mammalian host cell/tissue. To a considerable extent, this missing in-depth knowledge is due to the fact that historically, the investigation of metabolic alterations in the host and pathogen during infections posed major experimental challenges. Mass Spectrometry Imaging (MSI) is an analytical technique which can overcome some of these challenges. MSI add another dimension to metabolite profiling by providing in situ snapshots of the spatial distribution of biologically relevant metabolites in intact tissue sections. We sought to investigate the direct metabolic effects of bacterial or viral infection in mammalian tissues by employing matrix assisted laser desorption/ionization (MALDI-FTICR) Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry imaging. Brain tissue from an immunocompetent mouse model of Zika virus (ZIKV) infection which mirrors the neuropathophysiological properties of the virus in human hosts, and lung tissue from a mouse model of Francisella tularensis (Ft) infection was harvested from multiple animals at different times during infection. Sections from each tissue were cut and mounted on histology slides. Duplicate sections were utilized for in situ hybridization of ZIKV RNA in the case of the ZIKV infected brain sections, or Gram stained to detect bacterial lesions in Ft infected lung tissue. MSI revealed that the abundance of numerous metabolites, covering important metabolic pathways (Glycolysis, TCA cycle, lipid metabolism and fatty acid oxidation) were altered when compared to controls. Further, co-localization of brain sub-regions where ZIKV was actively replicating or areas of Ft colonization in the lung, allowed the direct examination of metabolite changes which occur at the host-pathogen interface. The data generated will be used in computational metabolic network reconstructions to provide a framework for the discovery of novel therapeutic targets or synergies and the prediction of key phenotypes of pathogenesis.

Matrix Assisted Laser Desorption Ionization Imaging Mass Spectrometry (MALDI IMS) of Drug-Induced Toxicity in a Zebrabfish Model


Determination of drug concentrations in tissue has historically been achieved using tandem mass spectrometry based platforms, however, without laborious dissections; traditional MS/MS does not offer any information concerning drug localization. Matrix assisted laser desorption ionization imaging mass spectrometry (MALDI IMS) is a label free approach in which the distribution of an analyte can be visualized across a tissue section in relation to the tissue’s histopathology. Zebrabfish (Danio rerio), are quickly becoming a popular model in toxicity studies due to their homogeneity with the human genome, small size, low cost, applicability to environmental studies and large breeding potential. Although a handful of MALDI IMS studies have been conducted in zebrafish, no one has assessed drug toxicity in a whole-body section, nor examined drug induced organ failure. Presented here is a pilot study in which a MALDI IMS method for the analysis of whole body zebrafish sections was developed. The method was further assessed in a zebrafish model dosed with 10mg/mL gentamicin. MALDI IMS images of both gentamicin and gentamicin metabolites will be presented, along with the overlap of drug distribution to tissue damage as an example of the power of this technology in further understanding drug induced toxicity.

MALDI IMS: Visualizing Pharmacologically Active Molecules “Breaking Bad” in Tissue

S. Castellino. GlaxoSmithKline, plc, King of Prussia, PA. Sponsor: L. Schnackenberg

Delivering safe and efficacious medicines is tied to our ability to understand the complex mechanistic relationships between molecular initiation events of pharmacologically active compounds and the cascade of biological consequences. The development and application of new technologies to investigate and interrogate biochemical events in tissue compartments is critical for unraveling the mechanistic pathways associated with drug-induced injury. Matrix-assisted laser desorption/ionization (MALDI) Imaging Mass Spectrometry (IMS) is an emerging technology which can determine the spatial distribution of a drug and its metabolites as well as endogenous compounds in tissue samples without the need for labeling. This methodology allows for the correlation of analyte tissue distributions with histology images, thereby integrating chemical structures with tissue morphology. MALDI IMS provides high spatial resolution (10µm), is highly sensitive, and can be quantitative. Furthermore, this imaging modality offers the potential to further our mechanistic understanding of drug disposition, disease progression and pharmacology including toxicity. This presentation will focus on our efforts to couple MALDI IMS and histology in drug development to better understand drug tissue disposition and gain mechanistic insights into drug correlated toxicities and efficacy. Case studies from early and late stage drug development, where MALDI IMS was employed to investigate the mechanisms of adverse events, provide insights into disposition, and PK/PD relationships will be presented.


R. R. Drake. Medical University of South Carolina, Charleston, SC. Sponsor: L. Schnackenberg

Glycoproteins account for approximately 80% of the proteins located at the cell surface and in the extracellular environment, and serve as binding ligands for cell adhesion, extracellular matrix molecules, signaling receptors, immune cells, lectins and pathogens. Alterations and changes in cell surface glyco- structure during carcinogenesis and other diseases have been documented, but the specifics in regards to which glycan and at what sites on glycoproteins are responsible for these changes is still poorly understood. Further, how glycan profiles and glycoproteins respond to therapeutics or xenobiotic exposures is even less characterized. To address this, we have recently optimized a MALDI mass spectrometry imaging method to spatially profile N-linked glycans in formalin-fixed paraffin-embedded (FFPE) tissue sections and tissue microarrays (TMAs). Tissues are incubated with a molecular coating of peptide N-glycosidase, and released N-glycans are detected using MALDI-FTICR imaging, linked directly with tissue histopathology. Two custom TMAs representing 20 different tumor types and other cancer models have been evaluated for systems glycomic comparisons. This in turn has generated a database of over 140 N-glycans derived from these tissues. Additional glycans representing different sialic acids detected in rodents, and ethylated derivatives of sialic acids are also included in the database. Using this methodology and data resources, the approach is proving informative for the comparative analysis of tissues in many model systems, including breast cancer drug resistance/sensitivity, gene knock outs of metabolic enzymes, tumor progression and patient derived tumor xenograft models. The goals are to link the disease or phenotypic changes identified for different N-glycan structural classes to metabolic, proteomic and genomic changes associated with each model.

Mechanisms and Effects of Diabetogenic Environmental Metals: Type II Diabetes mellitus and Diabetic Kidney Disease

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Complications arising from diabetes mellitus (DM) is the seventh leading cause of death in the US. Approximately 30.3 and 84.1 million Americans had type II DM or prediabetes, respectively, in 2015. The number of Americans with type II DM is projected to increase by nearly 20 million from 2015 to 2030. Lifestyle choices such as diet and exercise, in addition to genetics, are major factors in determining if someone will become type II DM. In addition to these factors, exposure to environmental metal and nonmetal substances (e.g., cadmium, arsenic, zinc, manganese, and selenium) may also play a significant role. Many epidemiological studies show a significant and positive association between exposure to toxic environmental metals and type II DM, prediabetes, or impaired fasting glucose. Experimental studies using animal models of metal toxicity show significant increases in fasting blood glucose levels or disruption of major mediators of metabolism. This session will examine the cellular and molecular mechanisms responsible for the diabetogenic effects of various metals. In addition, factors that may mitigate arsenic-induced dysglycemia, such as selenoproteins, will be discussed. Lastly, the session will review the synergistic or additive effects of cadmium-induced nephropathy in an in vitro model of diabetic nephropathy. This session will highlight the most recent experimental findings from presenters who are experts at the forefront of this field of study.
1051 Cadmium Accumulates within Pancreatic Islets at Levels Similar to the Renal Cortex in an Experimental Model of Long-Term Exposure

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Diabetes mellitus is a growing worldwide epidemic. Impaired pancreatic islet function is a hallmark of type 1 diabetes mellitus and for some, a key in the progression of type II diabetes mellitus. Multiple epidemiological and experimental studies show that exposure to the metal cadmium (Cd), is associated with diabetes mellitus and prediabetes. However, Cd is considered a classic nephrotoxicant that accumulates in the renal cortex at levels 8 to 20 times higher than any other tissue. The objective of this study was to quantify and compare the amount of Cd in the renal cortex to that of pancreatic islets. We also examined Zn because it is essential to normal pancreatic beta cell function and there is evidence that Cd and Zn may use the same transporters to gain entry into cells. Male Sprague Dawley rats were injected subcutaneously with either saline (control) or Cd (0.6 mg Cd/kg/day, 5 days per week). After 6 or 12 weeks of Cd treatment, kidney cortex tissue was collected and pancreatic islets were isolated. The freshly isolated islets were then either exposed to low (0.5 mg/ml) or high (3.0 mg/ml) glucose containing buffer for 4 hours. At the end of the incubation, islets were treated with lysing buffer and stored at -80 for later Cd and Zn determination. Kidney cortex tissue from 12 week treated animals had the highest Cd content at 11,007 ± 777 (nmol/g protein ± SE); in pancreatic islets isolated from 12 week Cd-treated animals and incubated in 0.5 mg/ml glucose the average Cd concentration was 7,305.2 ± 403. Surprisingly, at the same 12 weeks of Cd treatment there was significantly less Cd content in islets incubated at 3.0 mg/ml glucose compared to 0.5 mg/ml glucose. These results show that Cd accumulates within pancreatic islets to levels approaching that of the renal cortex. Overall, the study shows that pancreatic islets are sites of Cd accumulation, potential targets of Cd toxicity and likely involved in etiology of Cd-induced hyperglycemia.

1052 Methods of Investigating the Toxic Potential of Environmental Factors in Insulin-Producing Islets of Langerhans Illustrated Using the Example of Cadmium

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Failure of insulin-producing β-cells in islets of Langerhans in the setting of insulin resistance is the underlying cause for type 2 diabetes mellitus (T2DM). Genetic susceptibility, age, lifestyle, and environmental factors contribute to β-cell failure in T2DM. Environmental contributors towards β-cell failure in T2DM are underexplored. Herein we illustrate epidemiologic and experimental methods aimed at exploring the association between environmental factors and β-cell failure in the setting of T2DM using the example of cadmium (Cd). To that end, we first illustrate epidemiologic studies by us and others showing evidence for a diabetogenic effect of Cd using the NHANES database. Others and we previously provided evidence for a correlation between cadmium (Cd) exposure and T2DM as well as the accumulation of Cd in insulin producing β-cells. We report findings from our newly established murine oral Cd exposure model for the study of Cd-induced dysglycemia. For this, male C57BL/6N mice were exposed to CdCl2 or vehicle in drinking water. Obesity and insulin resistance was simulated by placing the animals on a high fat diet (42% calories from fat). We exposed animals to CdCl2 for 19 weeks followed by a 19-week washout period to yield an islet Cd concentration of 80.9 ± 9.3 nmol/g protein. This concentration was within the range of human islet Cd concentration, thereby representing a relevant islet Cd concentration. Blood Cd concentrations returned to near baseline levels at the end of the study. We found significant impairment of glucose clearance and reduced insulin secretion in standard in vivo glucose stimulated insulin secretion testing. In examining the differences in physiology between islets from mice exposed to Cd vs control mice, we found a lower ratio of insulin to proinsulin, pointing towards an impairment in normal insulin processing. These results point to an impaired processing of insulin precursors into mature insulin and the accumulation of misfolded insulin-a known contributor to islet dysfunction in the setting of T2DM. Results from next generation sequencing of mRNA from islets exposed to CdCl2 in vivo for 6 weeks via drinking water showed changes suggesting an increase in markers of inflammation but no changes in ER stress. This may suggest an attenuated response to the impaired insulin processing.

1053 Dissecting Mechanisms of Metal-Induced Beta Cell Dysfunction: Arsenic, Cadmium, Manganese, and Zinc

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Growing evidence suggests that exposures to environmental chemicals contributes to the current epidemics of diabetes. Several metals found in the environment have been implicated in diabetes risk, including inorganic arsenic (iAs), cadmium (Cd), manganese (Mn) and zinc (Zn). iAs and Cd are toxic heavy metals with no known physiological functions. On the other hand, Mn and Zn are essential micronutrients that play important roles in regulation of metabolic pathways and in antioxidant protection. Exposures to iAs and Cd have been consistently linked to compromised glucose homeostasis with impaired fasting glycemia or glucose tolerance, insulin resistance and/or beta cell dysfunction. In contrast, data on effects of Mn and Zn have been inconsistent. We used the rat insulinoma INS-1(832/13) beta cells, an established cell culture model in diabetes research, to compare the effects of iAs, Cd, Mn and Zn on glucose stimulated insulin secretion (GSIS) and on mechanisms involved in GSIS regulation, including mitochondrial metabolism and microRNA expression. We found that 24-hour exposures to non-cytotoxic concentrations of iAs, Cd and Mn inhibited GSIS, but only iAs impaired mitochondrial metabolism as demonstrated by decreased oxygen consumption rate, and by suppression of maximal respiration and spare respiratory capacity of mitochondria. Notably, no inhibition or activation of GSIS or mitochondrial metabolism was found in cells exposed to Zn. Exposure of INS-1(832/13) cells to iAs induced the expression of microRNA-146a, which is involved in beta cell function and survival, and suppressed expression of microRNA-217, which has been linked to apoptosis. Interestingly, exposures to Mn or Cd triggered the opposite response, decreasing microRNAs-146a expression and increasing expression of microRNA-217. Micro-RNA sequencing revealed a distinct small RNA profile associated with each metal exposure. Significant alterations were observed in the expression of a shared set of microRNAs with established targets along the insulin secretory pathway, as well as additional microRNAs unique to each metal exposure. These data suggest that iAs, Cd and Mn inhibit GSIS in beta cells; however, the mechanisms that underlie these effects may differ. Further analysis of microRNAs and their targets in beta cells may help to identify these mechanisms.

1054 Selenium and Selenoproteins Modulate Arsenic-Induced Metabolic Dysfunction


Diabetes is a devastating metabolic disease projected to afflict 693 million individuals worldwide by the year 2045. While caloric excess, physical inactivity, and genetic susceptibility all contribute to disease risk, these factors alone fail to fully account for the magnitude of the epidemic. Recently environmental toxicants acting as metabolism-disrupting chemicals (MDCs) have been implicated in the pathogenesis of diabetes. Based on the extent to which humans are exposed, one diabetes-linked MDC of significant importance is arsenic. Arsenic has been shown to disrupt the key pathways regulating glucose homeostasis, namely insulin secretion and insulin action, resulting in glucose intolerance in animal models. These effects are corroborated by epidemiologic evidence suggesting that arsenic exposure is associated with diabetes in human populations. Interestingly, arsenic and selenium have long been known to interact. Moreover, selenium-containing proteins (selenoproteins) have biological functions that are predicted to antagonize various aspects of arsenic toxicity. To test the hypothesis that selenoproteins protect against arsenic-induced metabolic dysfunction, male C57BL/6J mice were exposed to arsenic (sodium arsenite, As3+, 50 ppm) for 8-10 weeks on a selenium-deficient (0.01 ppm) or selenium-replete (0.1 ppm) diet. Body weight regulation and composition, glucose tolerance, and insulin secretion were examined. Identical studies were conducted in mice haploinsufficient for selenocysteine insertion sequence binding protein 2 (SBP2), a translation factor essential for the biosynthesis of all selenoproteins. Disruptions in either dietary selenium content and/or selenoprotein deficiency altered arsenic-induced effects on body composition and insulin secretion. These data suggest that selenium/ selenoproteins have mitigating role in arsenic-induced metabolic toxicity. Ongoing studies seek to define the specific selenoproteins mediating arsenic toxicity in order to both clarify the underlying mechanisms by which these factors preserve metabolic health as well as to identify populations who may be at enhanced risk of metabolic dysfunction from arsenic exposure.
Hyperglycemic Glucose and Cadmium Exposition: Two Loads Too Many for the Renal Proximal Tubule

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The progression of chronic kidney disease (prevalence > 3% of adult population) to end-stage renal disease is a source of considerable morbidity and mortality in western populations. While pathological processes of the glomerulus are known to play a major role in this disease, it is the demise of the renal tubules, predominantly the proximal tubule, that is the major correlate to the progression to end-stage disease. Though diabetes is the largest contributor to this disease, the heavy metal cadmium is increasingly appreciated as a significant etiological factor, and with respect to this metal, the proximal tubule is the main target site for renal toxicity. Proximal tubule cells are also a major site for glucose toxicity due to the major role of the proximal tubule in glucose reabsorption. During hyperglycemia, the proximal tubule-glucose load increases and potentially saturates the reabsorption process eventually leading to the accumulation of sorbitol, a toxic metabolic side-product of glucose produced through the reduction of the aldehyde group by aldose reductases. Since the exposure and accumulation of cadmium in the proximal tubule cells occurs throughout the human population, the development of chronic kidney disease occurs with a high base line of metal exposure, thus the development of this disease most likely occurs through and interaction or collaboration of these two agents. Our model for study are cultures of immortalized human proximal tubule cells and recently the newly developed, telomerase-immortalized human proximal tubule cell line, RPTEC/hTERT. We recently discovered that an isoform of aldose reductase is induced by cadmium exposure and we are now investigating the contribution of this enzyme to glucose-induced sorbitol accumulation and toxicity. Exposures to the glucose can also lead to a loss of epithelial character having similarities to the epithelial-to-mesenchymal transition, a process that might contribute to the interstitial fibrotic process seen in this disease. We have been characterizing this process, with respect to both morphology and gene expression. It can be appreciated that multiple factors contribute to toxicity and disease processes, and with respect to the kidney, cadmium and hyperglycemic glucose concentrations are two agents that can initiate and accelerate chronic kidney disease.

Pharmaceutical Investigative Toxicology: Case Studies in Optimizing Drug Discovery and Guiding Human Risk Assessment

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Toxicity and clinical safety have a major impact on drug development success. Moving toxicological studies into earlier phases of the research and development chain prevents drug candidates with a safety risk from entering clinical development. However, to identify candidates without such risk, safety has to be addressed proactively. Therefore, toxicology should ideally be integrated into the discovery process. Successful discovery phase drug safety assessment requires in-depth hazard identification and integrated experimental approaches to address target and lead compound risks to support target assessment. In order to identify a suitable candidate, selection, and derisking of safety flags from in vitro, animal, and clinical testing. Consequently, the application of innovative models and techniques that allow the identification of early hazards, prioritize chemical series, and steer chemical design and safety assessment should ideally be integrated into the early phases of the discovery process. Similarly, mechanistic insight into animal and clinical drug toxicities can be critical in developing informed human risk assessment. In this session, we will present case studies of innovative investigative discovery phase toxicology that have enabled mechanistic understanding and improved quantitative human risk assessment. Examples will include the use of recombinant proteins and antibodies to probe the effects of small molecule candidate drugs on in vivo safety biomarkers, use of 3D microphysiological models of human and animal organs, transcriptomics, drug metabolism, and modeling and simulation techniques to quantify translational risk assessment to humans.

Reduced Kupffer Cell Clearane Is Causing Elevation of Serum Toxicity Biomarkers in Rat and Monkey in Absence of Organ Injury

F. Pognon, Novartis, Basel, Switzerland.

Enzymatic serum biomarkers use to monitor drug safety in preclinical and clinical studies are sometimes not matching actual drug-induced organ injuries. Apparent toxicity biomarker elevations have been reported in animals treated with compounds affecting the proliferation and differentiation of Kupffer cells (KC), by inhibiting the macrophage colony-stimulating factor 1 (CSF1) pathway. Although KCs have been reported to play an important role in clearance of circulating enzymes, these co-incident observations have not formally established the causal link leading to elevated circulating biomarkers, as undetected organ damage could have been at the origin of this phenomenon. The purpose of the present studies was to explore the fate of exogenously injected histidine (His)-labeled recombinant enzymes ALT1 in rats treated with the CSF1 receptor antagonist BLZ945 and CKM (CK-MM isoform) in monkeys treated with MCS110, a CSF1-neutralizing monoclonal antibody. BLZ945 and MCS110 treatments resulted in elevations of endogenous serum ALT, AST or CK without causing histological detectable lesions or increase release of target organ specific micro RNAs (miRs). Additionally, both compounds were leading to a pharmacological reduction of KCs number. The elimination kinetics of injected His-tagged ALT1 and CKM were analyzed by non-comparmental (NCA) and a population analysis. Both analyses consistently demonstrated significant increase of the half-lives and delayed clearance rates of the His-tagged proteins after the BLZ945 and MCS110 treatment. The present data demonstrated that elevation of ALT and CK is independent of potential liver and muscle drug-induced toxicity by CSF1-pathway inhibitors, and likely resulting from reduced protein clearance by KCs in rats and monkeys.

Use of Early Phenotypic In Vivo Markers to Assess Human Relevance of an Unusual Rodent Non-Genotoxic Carcinogen In Vitro

A. Roth, F. Hoffman-La Roche Ltd, Basel, Switzerland.

Foci of altered hepatocytes (FAH) are considered putative, pre-neoplastic lesions that can occur spontaneously in aging rats or induced by chemicals or drugs. Progression of FAH to hepatocellular neoplasms has been reported repeatedly but increases in foci in rodents do not necessarily lead to tumors in carcinogenicity studies and the relevance for humans often remains unclear. Here we present the case of RG3487, a molecule which induced FAH and, later on, tumors in rats. Because the molecule was negative in genotoxicity assays it was classified as a non-genotoxic carcinogen. In order to assess the potential for liver tumor formation in humans, we analyzed treatment-induced changes in vivo to establish a possible mode of action (MoA). In vivo and in vitro gene expression analysis revealed that nuclear receptor signaling was unlikely to be the relevant MoA and no known mechanism could be established. We therefore took an approach comparing phenotypic markers, including mRNA changes, proliferation and glycogen accumulation, in vitro using models, including 3D MPS systems of different species to assess the human relevance of this finding. Since the alterations
observed in rats were not seen in the liver of mice or dogs in vivo, we could validate the relevance of the cell models chosen by use of hepatocytes from these species in vitro. This ultimately allowed for a cross-species comparison, which suggested that the formation of FAH and liver tumors was rat specific and unlikely to translate to human. Our work showed that phenotypic species comparison in vitro is a useful approach for assessment of the human relevance of pre-clinical findings where no known mechanism can be established.

1060 Characterization and Mechanistic Investigation of Hemolytic Anemia in Rats Induced by an Early Small Molecule Oncology Candidate

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To ensure our candidate molecules have an appropriate safety profile, Discovery & Investigative Toxicology aims to identify liabilities early and support chemistry in optimizing molecules for safety. In a 14-day rat toxicology study, our lead compound induced hemolytic anemia. Key pathological features included extravascular hemolysis accompanied by a regenerative bone-marrow response. The presence of Heinz bodies indicated oxidative damage to erythrocytes. Exposure of rat erythrocytes (RBCs) to the compound in vitro revealed no direct toxicity. In vivo met-ID revealed a chloroaniline metabolite previously shown to trigger hemolysis via hepatic conversion to an N-hydroxy derivative. The N-hydroxy, but not parent or aniline, triggered met-ID and oxidation of RBCs. Further evaluation of other molecules in the series revealed they all exhibited the same hemotoxic propensity. These early investigative toxicology studies enabled a rapid decision to be made to de-prioritise this series.

1061 Complex Cell Models and TK/TD Modeling and Simulation Guide Drug Discovery and Enhance Translational Safety Risk Assessment

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3D human cell culture and microtissue models can recapitulate tissue biology at a more physiological level compared to conventionally used 2D static cell cultures, enabling improved biological understanding of candidate drugs and their potential for toxicity. In addition, more quantitative safety risk assessments can be achieved when in vitro data is coupled to pharmacokinetic/pharmacodynamic (PK/PD) models, which allow translation of time-course and magnitude across biological systems, accounting for differences between in vitro and in vivo physiology. Here we explore the use of primary cell and microtissue models to provide translational safety risk assessment of oncology drug candidates. In the first case study we utilize 3D human airway in vitro models to dissect the mechanism of degenerative bronchiolar epithelium pathology observed in vivo with a kinase inhibitor lead series. In a second case study we evaluate the suitability of a 3D human gastrointestinal microtissue to model human GI adverse effects reported with a candidate drug, to investigate the underlying toxicity mechanism(s) as well as to model dosing schedules with improved tolerability for in vivo validation. A final case study will describe the development and use of a microfluidics system to replicate in vivo PK profiles within cell-based assays to enable improved in vivo translation of drug actions. This system has the potential to minimize animal studies for both efficacy and safety studies and effectively guide clinical use of candidate drugs.

1062 UVA Photosensitization of Melanin Induces Oxidative DNA Damage, and B-RAF Mutational Hotspots Are Major Targets

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Cutaneous malignant melanoma (CMM), the primary type of melanoma, occurs mainly at sunlight-exposed body regions, with 70% of CMM having B-Raf V600E mutations. It suggests that sunlight is a major CMM etiological agent that targets B-Raf codon 600. Intriguingly, the most frequent B-Raf V600E mutation ('GTG' to 'GAG') lacks UVB photo-damage signature; the exact mechanism of how sunlight induces melanogenesis remains unclear. In this study, we found that UVA induces a significant amount of oxidative DNA damage (ODD), particularly 8-oxo-deoxyguanosines (8-oxo-dG), in human melanocytes but not in non-pigmented normal human skin fibroblasts (NHSF). Also, UVA induces more 8-oxo-dG in African-American melanocytes (AAMC) than in European-American melanocytes (EAMC). In vitro, UVA sensitizes both eumelanin and pheomelanin to produce H2O2, which results in 8-oxo-dG induction. Mapping UVA-induced ODD in B-Raf, we found significantly more ODD in AAMC than in EAMC and discovered that both strands of codon 600 of B-Raf (AG-TGG-A/-TCA-TCT) are preferential sites for ODD formation. Furthermore, the ODD spectrum in B-Raf induced by UVA in melanocytes is the same as that induced by H2O2 in NHSF. We conclude that UVA sensitizes melanin to produce H2O2, which reacts with more genomic DNA to induce ODD. These results demonstrate that melanin plays a direct role in enhancing UVA-induced ODD formation and mutagenesis and that the B-Raf codon 600 is a major UVA target. We propose that UVA plays an important role in initiating melanogenesis.

1063 An Evaluation of the Cytotoxicity and Chromosome-Altering Effects of Vanadium Pentoxide and Sodium Metavanadate in Vitro

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Vanadium compounds are found in mineral ores and have become increasingly important in industrial manufacturing. Vanadium exists in several oxidation states with vanadium pentoxide (V2O5) and sodium metavanadate (NaVO3) being among the more common. Previous studies by the National Toxicology Program have shown that vanadium pentoxide is carcinogenic in rodents inducing alveolar/bronchiolar neoplasms. However, the mechanism for the tumor promoting effect of vanadium pentoxide is not known. This study will describe the development and use of a microfluidics system to replicate in vitro drug action and to distinguish toxicity from genotoxicity. In vivo studies revealed no direct toxicity. In vivo met-ID revealed a chloroaniline metabolite previously shown to trigger hemolysis via hepatic conversion to an N-hydroxy derivative. The N-hydroxy, but not parent or aniline, triggered met-ID and oxidation of RBCs. Further evaluation of other molecules in the series revealed they all exhibited the same hemotoxic propensity. These early investigative toxicology studies enabled a rapid decision to be made to de-prioritise this series.

1064 Determination of the Molecular Target of Aneugenic Compounds by a Multiplexed, Flow Cytometric Assay


An efficient in vitro method for determining genotoxic mode of action (MoA) is commercially available under the trade name MultiFlow®. Based on several DNA damage response biomarkers, this multiplexed assay characterizes compounds as clastogenic, aneugenic or nongenotoxic. Here, we report modifications to the base assay that extend its capabilities to include the determination of aneugenic agents’ molecular targets. Specifically, we have designed a follow-up assay that discriminates between the two most common mechanisms of aneugenicity: tubulin binding and off-target inhibition of mitotic kinases, especially Aurora kinases. For these experiments TK6 cells were exposed to each of 27 aneugens over a range of concentrations and in the presence of fluorescent taxol. The aneugens consisted of 12 mitotic kinase inhibitors and 15 diverse tubulin binders. After 4 hrs at 37°C cells were added to MultiFlow base solution (detergent, nucleic acid dye, RNase and counting beads) supplemented with fluorescent antibodies against phospho-histone H3 (p-H3) and Ki-67. The resulting detergent-activated nuclei were then analyzed by flow cytometry. Alterations in taxol-associated fluorescence were only observed in the case of the tubulin binders—increases in the case of stabilizers, decreases for destabilizers. Pan-Aurora kinase B-specific inhibitor was the only agents that dramatically decreased the ratio of p-H3-positive to Ki-67-positive nuclei. Unsupervised hierarchical clustering based on taxol fluorescence and p-H3/Ki-67 ratios clearly distinguished 9/9 compounds with pan- or Aurora B-inhibiting activity, 12/12 tubulin destabilizers, and 3/3 tubulin stabilizers. These data demonstrate the adaptability of the MultiFlow platform to address not only genotoxic MoA, but also to elucidate specific molecular targets responsible for aneugenic...
Pig-a assay is a sensitive flow cytometry-based immunophenotypic method for detecting cells that have mutations in the endogenous X-linked Pig-a reporter gene. Instrumentally, phenotypically mutant cells measured in the assay are deficient in protein markers tethered to the cellular surface via glycosyl phosphatidylinositol (GPI) anchors. Pig-a assays can be established for nucleated cells and for nucleic acids-free red blood cells (RBCs) of various species. Rodent RBC Pig-a assay is useful in non-clinical safety evaluations of novel drugs; a regulatory-compliant test guideline for performing and interpreting the RBC Pig-a assay is under development. An outstanding issue for the RBC Pig-a assay was proving that the assay detects what it claims to detect, i.e., mutation in the Pig-a gene. We developed a Pig-a assay for precursors of rat RBCs, bone marrow erythroid cells (BMEs), and for lineage-related bone marrow granulocytes (BMGs). In rats treated with a prototypical mutagen, 7,12-dimethyl benz(a)anthracene (DMBA), the frequencies of phenotypically mutant marker- and GPI anchor-deficient BMEs and BMGs are increased. We sorted out the marker-deficient mutants in bulk and analyzed the Pig-a gene in them using next generation sequencing methodology. We determined that sorted BMEs have mutations in the Pig-a gene: mostly base-pair substitutions at dA and dG on the non-transcribed strand of genomic DNA, with dA to dT transversion being the predominant mutation. Such a spectrum is consistent with mutations induced by DMBA in vivo in other cell types (e.g., in the Pig-a and Hprt genes of rat T-cells). Recently, a similar relationship between the mutant phenotype and the spectrum of mutations in the Pig-a gene has been demonstrated for BMEs and BMGs from rats treated with another prototypical mutagen, N-ethyl-N-nitrosourea (ENU). ENU induced a different spectrum of mutations, mostly dt to dA transversions on the non-transcribed DNA strand. Mutant RBCs measured in the RBC Pig-a assay arise from mutant BMEs. Since phenotypically mutant BMEs in rats treated with two different compounds have agent-specific Pig-a mutations, the RBC Pig-a assay should measure true Pig-a mutant cells induced by agents of interest. Thus, the RBC Pig-a assay detects what it claims to detect, mutation in the Pig-a gene. The analysis of the spectrum of DMBA-induced rat BMG Pig-a mutations (currently in-progress) will further support such a claim.

\[P1\] 1065 Spectrum of DMBA-Induced Pig-a Mutations in Rat Bone Marrow Erythroid Cells

Pig-a assay is a sensitive flow cytometry-based immunophenotypic method for detecting cells that have mutations in the endogenous X-linked Pig-a reporter gene. Instrumentally, phenotypically mutant cells measured in the assay are deficient in protein markers tethered to the cellular surface via glycosyl phosphatidylinositol (GPI) anchors. Pig-a assays can be established for nucleated cells and for nucleic acids-free red blood cells (RBCs) of various species. Rodent RBC Pig-a assay is useful in non-clinical safety evaluations of novel drugs; a regulatory-compliant test guideline for performing and interpreting the RBC Pig-a assay is under development. An outstanding issue for the RBC Pig-a assay was proving that the assay detects what it claims to detect, i.e., mutation in the Pig-a gene. We developed a Pig-a assay for precursors of rat RBCs, bone marrow erythroid cells (BMEs), and for lineage-related bone marrow granulocytes (BMGs). In rats treated with a prototypical mutagen, 7,12-dimethyl benz(a)anthracene (DMBA), the frequencies of phenotypically mutant marker- and GPI anchor-deficient BMEs and BMGs are increased. We sorted out the marker-deficient mutants in bulk and analyzed the Pig-a gene in them using next generation sequencing methodology. We determined that sorted BMEs have mutations in the Pig-a gene: mostly base-pair substitutions at dA and dG on the non-transcribed strand of genomic DNA, with dA to dT transversion being the predominant mutation. Such a spectrum is consistent with mutations induced by DMBA in vivo in other cell types (e.g., in the Pig-a and Hprt genes of rat T-cells). Recently, a similar relationship between the mutant phenotype and the spectrum of mutations in the Pig-a gene has been demonstrated for BMEs and BMGs from rats treated with another prototypical mutagen, N-ethyl-N-nitrosourea (ENU). ENU induced a different spectrum of mutations, mostly dt to dA transversions on the non-transcribed DNA strand. Mutant RBCs measured in the RBC Pig-a assay arise from mutant BMEs. Since phenotypically mutant BMEs in rats treated with two different compounds have agent-specific Pig-a mutations, the RBC Pig-a assay should measure true Pig-a mutant cells induced by agents of interest. Thus, the RBC Pig-a assay detects what it claims to detect, mutation in the Pig-a gene. The analysis of the spectrum of DMBA-induced rat BMG Pig-a mutations (currently in-progress) will further support such a claim.

\[P2\] 1066 Similarities in the Transcriptomic Signatures in the Duodenum of Mice Exposed to Hexavalent Chromium, Captan, or Folpet Inform Mechanisms of Chemical-Induced Mouse Small Intestine Cancer
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Shared key events observed in intestinal cancers in mice exposed orally to hexavalent chromium (Cr(VI)) and the fungicides captan or folpet are the basis for similar non-motivated modes of action. Based on comparable histopathological findings in the duodenum (e.g. villus enterocyte cytotoxicity and crypt epithelial hyperplasia). Based on these similarities, we hypothesized that transcriptomic responses in the duodenum are also similar across these three agents. Such similarities at the molecular level would support a common adverse outcome pathway (AOP) for chromium and fungicides. A key hypothesis by evaluating transcriptomic responses in the duodenum tissues of B6C3F1 mice exposed to either Cr(VI) (180 ppm), drinking water), captan or folpet is whether there will be gene expression profiles similar between agents, with overall Pearson correlation coefficients >0.6. Second, gene-specific overlap comparisons revealed that 126/546 (23%) differentially expressed genes were altered in the same direction across all three agents. Third, gene set enrichment analysis was conducted using a broad range of curated gene sets, with 39 (23%) gene sets closely modulated between Cr(VI) and captan or folpet. A total of 25 upregulated gene sets were modulated by all three compounds, which were related to cellular metabolism, stress, inflammatory/immune cell response, and cell proliferation, including upregulation in HIF1- and AP1-signaling pathways (which are related to intestinal injury and angiogenesis/carcinogenesis). Transcriptomic profiles also differed between crypt and villus cells, indicating that alterations at the gene expression level are cell-type-specific. There was a lack of enrichment of DNA damage response-related gene sets. The similarities in Cr(VI)-, captan-, and folpet-induced effects at the molecular level support an AOP for mouse intestinal cancer that involves cytotoxic mechanisms.

\[P3\] 1067 Mammary Tumor Prevention Effects of Broccoli-Derived Sulforaphane in Rats Exposed to 17β-Estradiol Is Mediated by Multiple Cytoprotective Mechanisms
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Although estrogens play a vital role in health and aging, elevated estrogens have been recognized as an important contributor to breast cancer risk. The receptor-independent, oxidative metabolism of estrogens is an important pathway for estrogen-mediated carcinogenesis that facilitates the formation of DNA-damaging adducts. In healthy cells, the formation of these adducts are controlled by cytoprotective enzymes such as catechol-O-methyltransferase (Comt) and NADPH:quinone oxidoreductase 1 (Nqo1). Pharmacologic activators of the Nrf2 signaling pathway such as broccoli-derived sulforaphane (SFN) have been shown to activate these enzymes. Further, SFN can also alter cellular metabolic pathways leading to cytoprotection. Thus, we hypothesized that administering SFN to animals exposed to 17β-estradiol (E2) would prevent mammary tumor formation via altered metabolism and reduced DNA damage. In our study, 4-6 week old female August Copenhagen Irish rats were implanted with E2 pellets (3 mg x 3) and were gavaged with either DMSO or 100 μmol/kg SFN for 56 weeks. The Kaplan-Meier curve showed that SFN-treated rats were significantly protected against mammary tumor formation compared to DMSO. Serum free fatty acid and triglyceride species were ~2-fold lower in SFN-treated rats. The mammary glands of SFN-treated rats showed ~2-fold higher Nqo1 and Comt as well as significantly lower Fatty acid synthase and Acetyl-CoA carboxylase 1 transcripts. SFN treatment also lead to ~2-fold lower phosphorylated γ-H2a.x and altered expression of DNA damage response markers in the mammary gland. This study indicated that SFN potently protects against breast cancer through multiple mechanisms in a clinically relevant chemical carcinogenesis model. Funding: Breast Cancer Research Foundation and NIH R35CA197222.

\[P4\] 1068 Aflatoxin B1, Induces Fibrosis and Cirrhosis in Nrf2 Knockout Rats
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Aflatoxin B1 (AFB1), a mycotoxin produced by certain molds that contaminate foods, is a cause of liver cancer in humans. AFB1-induced hepatocarcinogenesis in rats is predicted by the focal burden of the glutathione S-transferase P (GSTP)-positive foci in livers well before cancers develop. GSTP gene expression is regulated by the transcription factor Nrf2. It has been reported that Nrf2 is constitutively activated in many kinds of human cancers. To examine roles of Nrf2 in AFB1-induced hepatocarcinogenesis, we dosed wild-type and Nrf2 knockout (Nrf2 KO) rats repeatedly with AFB1 (150 μg/kg/day, 5 d/week) for 12 weeks, followed by a one-month respite and then 2 weeks of 100 μg/kg/day, 5d/wk). Almost half of Nrf2 KO rats were sensitive to AFB1 and died between weeks 3 and 4; this dosing regimen had no effect on survival in wild-type rats. All surviving animals were sacrificed at 21 weeks. As reported previously, AFB1 induced substantial numbers of GSTP-positive foci in wild-type rats. However, GSTP-positive foci did not appear in the surviving Nrf2 KO rats, although bile duct cells and a few emerging single cells were positive for GSTP staining. Thus, GSTP expression in hepatocytes appears to be Nrf2-dependent. All surviving Nrf2 KO rats at 21 weeks showed cirrhosis in which most of the hepatocytes had disappeared. Some Nrf2 KO rats lived showed regenerative nodules surrounded by fibrosis. No fibrosis or cirrhosis was observed in the liver of wild-type mice at this timepoint. Clearly, Nrf2 plays a strong protective role against AFB1-induced liver injury. However, in AFB1-exposed Nrf2 KO rats, hepatocytes are damaged and disappear, leading to fibrosis and cirrhosis. Historically, it is well known that cirrhosis is not part of the etiopathogenesis of AFB1-induced hepatocarcinogenesis in wild-type rats. Whether chronic very low dose administration of AFB1 to Nrf2 KO rats could induce hepatocarcinogenesis driven through cirrhosis remains unknown. This research was supported by Fund for the Promotion of Joint International Research, JSPS KAKENHI 15KX0325, in Japan.
1069 Adverse Outcome Pathway of Ionizing Radiation Leading to Increased Risk of Breast Cancer

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Knowledge about established breast carcinogens can support improved 21st century toxicological testing methods by identifying key mechanistic events. Ionizing radiation (IR) increases the risk of breast cancer, especially for women and for exposure at younger ages. We used the Adverse Outcome Pathway (AOP) framework to outline and evaluate the evidence linking ionizing radiation with breast cancer from molecular initiating events (MIE) to the adverse outcome (AO) through intermediate key events (KE). We identified prospective key events using recent literature on ionizing radiation and carcinogenesis, focusing on review articles. We searched PubMed for each key event and ionizing radiation, and used references cited in the resulting papers and targeted searches with related key words to identify additional papers. We manually curated publications and evaluated data quality. The AOP specifies that ionizing radiation directly and indirectly causes DNA damage and increases production of reactive oxidative and nitrosative species (RONS), and these are designated as MIEs. RONS lead to DNA damage (MIE) and both lead to mutations (KE) and proliferation (KE) leading to the AO, but RONS and DNA damage also increase inflammation (KE). Inflammation contributes to direct and indirect effects (effects in cells not directly reached by IR) via positive feedback to RONS and DNA damage, and separately increases the AO through pro-carcinogenic effects on cells and tissue. These MIEs and KEs overlap at multiple points with events characteristic of “background” induction of breast carcinogenesis, including hormone-responsive proliferation, oxidative activity, and DNA damage. These overlaps make the breast particularly susceptible to ionizing radiation and reinforce the importance of these MIEs and KEs as part of toxicological panels for carcinogenicity. The AOP identifies areas for additional research, including better description of the time and dose-dependence of MIEs and KEs in many tissues directly and indirectly exposed to IR. The AOP will inform development of new assays, and offers new applications and context for existing assays addressing these KEs. Ultimately, this AOP will improve methods that predict chemical breast carcinogens so that exposure can be reduced.

1070 Phosphatidic Acid Enhances Liver Regeneration after Acetaminophen Hepatotoxicity by Promoting Phosphorylation and Inhibition of GSKβ3

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Acetaminophen (APAP) overdose is the primary cause of acute liver failure (ALF) in the US. The current treatment is N-acetylcysteine (NAC). However, NAC is only effective at ≤16h post-overdose, and approximately half of all patients present later. Clearly, new treatments are needed. One approach is to enhance liver regeneration. We recently discovered that phosphatidic acid (PA) promotes liver regeneration, but the mechanism is not known. The Wnt-GSKβ3-β-catenin signaling pathway is known to be important for regeneration after APAP overdose. Thus, we hypothesized that PA activates that pathway. Male C57BL/6J mice were treated with 300mg/kg APAP (i.p.) followed by vehicle (Veh) or 20mg/kg FSG67 (PA synthesis inhibitor) 2, 24, and 48h later. Some mice received 600 nmol of the GSK3 inhibitor L803-mts in addition to APAP and FSG67. Liver and blood were harvested at 0, 6, 24, and 52h post-APAP. Liver injury (plasma ALT, histology) and regeneration markers (PCNA, Cyclin D1) were measured, as well as Wnt, GSKβ3 and β-catenin. FSG67 post-treatment reduced PCNA protein (mean±2SE. 10±1 vs. 7±0.7 fold over 0 h for Veh vs. FSG67, respectively). Wnt and β-catenin increased. GSKβ3 phosphorylation (which inhibits GSKβ3 and promotes regeneration) was almost completely prevented in the FSG67 group at 24h post-APAP (26±9 vs. 3±1 fold over 0 h for Veh vs. FSG67). To confirm that loss of PA affects regeneration through increased GSKβ3 activity, we performed a rescue experiment in which mice were co-treated with FSG67 and the GSK3 inhibitor. Messenger RNA for PCNA (1±0.2 vs. 0.6±0.2, Veh vs. FSG67) and the β-catenin target cyclin D1 (1±0.1 vs. 0.3±0.2, Veh vs. FSG67) decreased with FSG67 treatment, but were restored by the GSK3 inhibitor (0.8±0.1 and 1.3±0.2 for PCNA and cyclin D1 mRNA, respectively). PA enhances regeneration through Wnt-independent effects on GSKβ3 and β-catenin. Additional research is needed to determine how PA alters GSKβ3 phosphorylation. Support: AASLD Foundation and NIGMS T32 GM106999.

1071 De Novo Fibrinogen Synthesis Promotes Liver Repair after APAP Overdose in Mice

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Acetaminophen (APAP) overdose is the leading cause of acute liver failure (ALF) in the United States. The coagulation protein fibrinogen relocates from plasma to the injured liver after APAP overdose, and hepatic fibrinogen (fibrinogen) deposits promote liver repair. Induction of de novo fibrinogen expression is also evident in APAP-challenged liver. Whether fibrinogen induction regulates plasma fibrinogen levels and liver repair after APAP overdose is unknown. We hypothesized that hepatic fibrinogen induction restores plasma fibrinogen after APAP overdose. To test this hypothesis, we used an N-acetylgalactosamine (GalNAc)-conjugated fibrinogen β (Fgbβ)-targeted anti-sense oligonucleotide (ASO). C57BL/6j mice were pretreated with GalNAc-Fgb-ASO or GalNAc-control-ASO (30 mg/kg) 24 hours prior to administration of APAP (300 mg/kg). Coincident with increased hepatic necrosis (38.6%), plasma fibrinogen levels were reduced and hepatic fibrinogen deposits were evident 24 hours after APAP challenge in mice given control-ASO. By 48 hours, plasma fibrinogen levels were restored and liver repair was evident (6.4% necrosis). Fgb-ASO-pretreated mice had equivalent plasma fibrinogen levels and were unaffected by APAP induced necrosis. To confirm that loss of PA affects regeneration through Wnt-independent effects on GSKβ3 and β-catenin. Additional research is needed to determine how PA alters GSKβ3 phosphorylation. Support: AASLD Foundation and NIGMS T32 GM106999.
Hepatic in vitro systems require phenotypic characteristics of liver cells which mimic those in vivo in humans. In addition to parenchymal hepatocytes, non-parenchymal cells (NPCs) accounting for approximately 30% of the total liver cell population, prominently contribute to the progression of liver diseases. Here, 3D primary human hepatocyte (PHH) spheroids were generated using Corning ULA plates (Bell et al., 2016) with or without the addition of crude NPC fractions (Baze et al., 2018) to produce co-culture spheroids from both matching and non-matching PHH and NPC pairs (6 PHH and 5 NPC donors). Phenotypic characterization of hepatocyte-specific markers in the co-culture spheroids revealed abundant expression of CYP3A4 and albumin at levels comparable to monoculture spheroid counterparts. Analyses of the cellular composition of the spheroid co-cultures at the mRNA level showed increased expression of mRNA markers for Kupffer cells (CD68, CD163), hepatic stellate cells (Cytogobulin, Vimentin, Vinculin) and liver sinusoidal endothelial cells (PECAM1) compared to corresponding parenchymal hepatocyte monocultures. Incorporation of NPCs was further confirmed by immunostaining which demonstrated variable incorporation of NPCs based on specific donors and PHH-NPC pairings. The plasticity of monoculture and co-culture spheroids was evidenced by time-dependent changes in the mRNA expression of NPC markers over time. Co-cultures demonstrated potential applications in modelling liver diseases as evidenced by increases in the response to IL-6 following lipopolysaccharide stimulation and increases in LOX and COL1A1 expression in response to transforming growth factor β (TGF-β) which could be negated in the presence of a TFG-β receptor inhibitor. High concentrations of free-fatty acids (480 µM) were also able to induce stress responses and pro-fibrogenic expression of COL1A1 almost exclusively in co-culture spheroids. In conclusion, a time course of 3D spheroid co-cultures of PHH combined with crude NPC fractions was characterized. This indicated dependence on suitable donor combinations to form and maintain co-culture spheroids and the requirement for NPCs to induce features related to pro-fibrotic outcomes.

An in vitro system requires phenotypic characteristics of liver cells which mimic those in vivo in humans. In addition to parenchymal hepatocytes, non-parenchymal cells (NPCs) accounting for approximately 30% of the total liver cell population, prominently contribute to the progression of liver diseases. Here, 3D primary human hepatocyte (PHH) spheroids were generated using Corning ULA plates (Bell et al., 2016) with or without the addition of crude NPC fractions (Baze et al., 2018) to produce co-culture spheroids from both matching and non-matching PHH and NPC pairs (6 PHH and 5 NPC donors). Phenotypic characterization of hepatocyte-specific markers in the co-culture spheroids revealed abundant expression of CYP3A4 and albumin at levels comparable to monoculture spheroid counterparts. Analyses of the cellular composition of the spheroid co-cultures at the mRNA level showed increased expression of mRNA markers for Kupffer cells (CD68, CD163), hepatic stellate cells (Cytogobulin, Vimentin, Vinculin) and liver sinusoidal endothelial cells (PECAM1) compared to corresponding parenchymal hepatocyte monocultures. Incorporation of NPCs was further confirmed by immunostaining which demonstrated variable incorporation of NPCs based on specific donors and PHH-NPC pairings. The plasticity of monoculture and co-culture spheroids was evidenced by time-dependent changes in the mRNA expression of NPC markers over time. Co-cultures demonstrated potential applications in modelling liver diseases as evidenced by increases in the response to IL-6 following lipopolysaccharide stimulation and increases in LOX and COL1A1 expression in response to transforming growth factor β (TGF-β) which could be negated in the presence of a TFG-β receptor inhibitor. High concentrations of free-fatty acids (480 µM) were also able to induce stress responses and pro-fibrogenic expression of COL1A1 almost exclusively in co-culture spheroids. In conclusion, a time course of 3D spheroid co-cultures of PHH combined with crude NPC fractions was characterized. This indicated dependence on suitable donor combinations to form and maintain co-culture spheroids and the requirement for NPCs to induce features related to pro-fibrotic outcomes.

Environmental disasters such as the flooding that impacted Houston, Texas after Hurricane Harvey may lead to the redistribution of contaminants from both sediment as well as industrial/hazardous waste sites, resulting in exposure and potential health risks to residents who live in close proximity. Traditional hazard identification methods are not suitable to determine the chemical composition and potential hazard of the exposures to hazardous mixtures after environmental emergency events. Therefore, new methods are urgently needed to enable faster responses to emergency events. This study aimed to test whether a panel of physiologically-relevant human cell populations, prominently contributing to the progression of liver diseases, can be used to identify key events and molecular mechanisms used to understand the underlying key events and molecular mechanism used to develop putative AOPs for hepatic lipid dysfunction. This abstract does not necessarily reflect US EPA policy.

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Addressing inter-individual variability is a critical but challenging step in health risk assessments. Most environmental toxicants, including tetrachloroethylene (PERC), lack experimental data on inter-individual variability, hence default uncertainty factors are applied to account for inter-individual variability in toxicokinetics (UF Toxicokinetics (UF TK) = 2.43-3.51) and toxicodynamics (UF Toxicodynamics (UF TD) = 3.16). One of the important contributors to inter-individual variability in toxicity of chemicals is variability in their metabolism. Specifically, kidney toxicity of PERC has been associated with generation of glutathione conjugation metabolites; however, a low flux of PERC metabolism through glutathione conjugation (<0.5%) has raised concern on this mode of action in vivo. In this study, we aimed to better characterize the variability in glutathione conjugation and kidney toxicity of PERC and their underlying molecular mechanisms by using the Collaborative Cross (CC) mouse population. Male mice from 45 CC strains were intragastrically dosed with PERC (1000 mg/kg) or vehicle (5% Alkamuls-EL 620 in saline) and euthanized at various time points up to 12 hours (n=1/strain/time). Concentration-time profiles showed wide variability among strains in toxicokinetics of S-(1,2,2-trichlorovinyl)glutathione (TCVG, UF TK = 1.35-2.66) among liver, kidney and serum, S-(1,2,2-trichlorovinyl)-L-cysteine (TCVC, UF TK = 2.43-3.51), and N-acetyl-S-(1,2,2-trichlorovinyl)-L-cysteine (NACTCVC, UF TK = 2.62-3.25) in mouse serum, liver, and kidney. Acute treatment with PERC (24 hrs) had significant effects (paired t-test, p<0.05) on kidney weight loss, increased kidney to body weight ratio, induction of fatty acid metabolism-associated genes (Acot1, Fabp1, and Ehhadh), and markers of proximal tubular injury (expression of Kim1/Havn1). Expression of Kim1 in kidney was positively correlated with formation of TCVG in liver (r=0.60). In conclusion, our results demonstrate that the default uncertainty factor for toxicokinetic variability may be marginally adequate to protect 95% of the population for kidney toxicity mediated by PERC. Further refinement of the characterization of inter-individual variability can be accomplished by incorporating these data into in silico population models both for toxicokinetics (such as a physiologically-based pharmacokinetic model) as well as for toxicodynamic responses.
1077 mRNA-Sequencing Identifies Liver as a Potential Target Organ for Triphenyl Phosphate in Embryonic Zebrafish

Triphenyl phosphate (TPHP) is a commonly used organophosphate flame retardant and plasticizer in the United States. Over the past decade, there has been a marked increase in the use of TPHP due to the phase-out of certain brominated flame retardants. Using zebrafish as a model, previous studies have shown that TPHP exposure from 24 to 72 hours post fertilization (hpf) results in severe cardiac looping defects by 72 hpf - a phenotype that is dependent on exposure during pharyngula (24-48 hpf) and mitigated by pre-treatment with non-toxic concentrations of a pan-retinoic acid receptor (RAR) agonist (fenretinide). Therefore, the objectives of this study were to 1) rely on mRNA-sequencing to identify pathways before and after cardiac looping (30 and 48 hpf, respectively) that may be impacted following exposure to 10 µM TPHP from 24-48 hpf and 2) determine whether pre-treatment with 2 µM fenretinide from 24-30 hpf mitigates cardiotoxicity-related pathways within embryos exposed to TPHP from 30-48 hpf. Based on mRNA-sequencing, TPHP exposure from 24 to 30 hpf and 24 to 48 hpf significantly affected the abundance of 305 and 274 transcripts, respectively, relative to vehicle (0.1% DMSO) controls. In addition, minor effects on cardiotoxicity- and nephrotoxicity-related pathways, Ingenuity Pathway Analysis (IPA) of significantly affected transcripts from 30- and 48-hpf embryos revealed that hepatotoxicity-related pathways were strongly affected following exposure to TPHP alone. Moreover, while pre-treatment with fenretinide mitigated TPHP-induced cardiac looping defects at 72 hpf, IPA revealed that fenretinide was unable to block TPHP-induced effects on hepatotoxicity-related pathways at 30 and 48 hpf, suggesting that, unlike the heart, TPHP-induced hepatotoxicity may be RAR-independent. Overall, our mRNA-sequencing-based data suggest that, in addition to the heart, the embryonic liver may be highly susceptible to TPHP exposure during early development. Therefore, our ongoing studies are focused on confirming that TPHP exposure alters the normal trajectory of liver development (based on phenotypic data) within zebrafish embryos and 2) identifying the mechanism of action that leads to potential TPHP-induced effects on hepatocytes within zebrafish and human cell-based systems.

1078 The Cross-Laboratory Testing and Comparison of a Liver Microphysiological System
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Drug-induced liver injury (DILI), and a poor understanding of human pharmacokinetics have long been cited as the major reasons for drug failure in clinical trials or post-marketing. This is in part due to the limitations of existing animal and in vitro models to accurately predict drug toxicity and efficacy in humans. To address these limitations, microphysiological system (MPS) models of the liver are being actively developed; however, the adoption of these models, with 3D cultures? exploration of best practices with human hepatocyte models over time in culture

1079 Acute In Vitro Nephrotoxicity of Three Brominated Flame Retardants
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Brominated flame retardants (BFRs) are a class of organohalogens commonly added to commercial products such as computers, electronics, textiles, and furniture to reduce their flammability. BFRs have significant environmental persistence and are reported to be detected in human blood and breast milk. In particular, tetrabromobisphenol A (TBBPA), hexabromocyclododecane (HBCD), and polybrominated diphenyl ethers (PBDEs) occupy 17 percent of the global flame retardant market and have been reported to have adverse effects on humans and wildlife. As such, the mechanisms of BFR-induced toxicity are actively being explored under the US EPA Toxic Sub stance Control Act. Previous research shows that these compounds cause nephrotoxicity in rats and mice; however, the mechanisms mediating this nephrotoxicity are unknown. In the present study, we determined the effects of TBBPA, HBCD, and BDE 47 on cell viability in rat (NRK), human embryonic (HEK-293), and adult human (HK-2) kidney cells after 48 hours. We observed a concentration- and species-dependent effect on MTT staining, with IC50 values of 58, 38, and 3 µM for TBBPA; 20, 16, and 7 µM for HBCD; and 46, 40, and 14 µM for BDE 47 in NRK, HEK-293, and HK-2 cells, respectively. We assessed the mechanisms of cell death by measuring annexin V staining and propidium iodide (PI) staining as markers of apoptosis and necrosis, and by studying changes in nuclear morphology via DAPI staining. Significant increases in both the percentage of annexin V- positive cells and the percentage of annexin V and PI double-positive cells suggested that all compounds were inducing apoptosis. These data suggest that BFRs induce species-dependent toxicity in renal cells, with higher levels of toxicity being induced in human cells. The mechanisms mediating this differential toxicity is the subject of future studies.

1080 What’s So Special about 3D Spheroid Cultures? Exploration of Best Practices with Human Hepatocyte Models over Time in Culture
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Advances in organotypic, three-dimensional (3D) cell culture models for human liver hold great promise for high throughput toxicology screening due to their enhanced physiological relevance. However, the development and evaluation of these models for predictive toxicology research has relied largely upon basic assumptions borrowed from two-dimensional (2D) hepatocyte culture systems (e.g., high glucose media, corticosteroids, fetal bovine serum), with insufficient characterization of the impact of these factors on spheroid maturation and stability over time. To address these shortcomings, we investigated the dynamics of metabolic competence and gene expression of 3D HepaRG spheroids over time, and compared transcriptomic profiles between 2D and 3D culture models to explore distinguishing molecular characteristics of 3D HepaRG spheroids. Our findings revealed the enzymatic activities of major drug metabolizing enzymes, including CYP3A4, initially decay over time in 3D culture with a half-life of ~12-24 h during cell aggregation. As spheroids mature from this “developmental” liver state over time, enzymatic activities rise and eventually exceed their initial 2D-differentiated levels after ~7-10 days in culture. Comparing transcriptomic signatures with mature 3D spheroids to their differentiated and proliferated 2D comparators revealed characteristic cellular pathways, including glycolysis and fatty acid metabolism. Bioenergetic differences were functionally apparent between 2D and 3D models, with 3D models exhibiting increased oxidative metabolism over time in culture and shifting away from glycolytic 2D cultures. Further exploration using varied culture media composition (e.g., serum, glucose, corticosteroid) revealed unique requirements with 3D spheroids for optimal maturation, stability, and reduced variability. To characterize how these factors influence liver modeling, high throughput transcriptomics and cell viability assays were performed in response to exposures with a focused panel of prototypical liver effectors, including menadione, tamoxifen, aflatoxin B1, rifampicin, omeprazole, phenobarbital, and acetaminophen. Altogether, our findings identified key factors important for organotypic, 3D spheroid models and establish best practices for their use in predictive toxicology screening.
Air pollution, particularly smoke produced from biomass combustion, is pneumotoxic. However, the mechanisms underlying the toxic effects of these materials on lung epithelial cells is not fully understood. Transient receptor potential (TRP) ion channels are a family of proteins that mediate some of the pro-inflammatory and cytotoxic effects of air pollutants, typically via disruption of intracellular calcium homeostasis. We hypothesized that TRP ion channels that are activated by wood smoke particles might regulate cytocytotoxicity in lung epithelial cells treated with wood smoke particles via mechanisms involving disruption of intracellular calcium homeostasis; specifically causing endoplasmic reticulum stress and E1FαK3/PERK-dependent signaling. Using fluorescent calcium imaging and various human TRP channel expression assays, it was found that TRPV3 resides on the endoplasmic reticulum of human lobular bronchial epithelial cells. Consistent with the induction of endoplasmic reticulum stress and E1FαK3/PERK activation in lobar cells, treatment with pine wood smoke particles promoted the time-dependent induction of pro-apoptotic DDIT3 and ATF3, biomarkers of E1FαK3/PERK activation during ER stress. As above, both TRPV3 and TRPA1 antagonists substantially attenuated these responses. Collectively, these data show that activation of TRPV3 and TRPA1 by wood smoke particles triggers ER stress in primary human lung epithelial cells, and that these events contribute to the acute cytotoxic effects of pine wood smoke particles on bronchial epithelial cells. These findings expand our understanding of how wood smoke adversely affects the lung.

**1084 Air Quality: An Interface between Environment, Climate Change, and Public Health**

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In recent years, Imperial County has consistently ranked as the top California County with the highest asthma rate in children. Community members are concerned their breathing problems are due to one or more of many sources of pollution that contaminate Imperial Valley (IV). Of particular interest is the potential toxicity of particles rising from the Salton Sea. With polluted runoff as the only water source, the Salton Sea has become increasingly polluted and is rapidly shrinking. No one has differentiated the sources of particulate matter (PM) in IV or their connection to asthmatic symptoms. Our goal is to investigate the differences between, and potential harmfulness of airborne particles to which IV residents are exposed. A state-of-the-art mobile sampling unit has been designed to collect PM of various size fractions, from ultrafine (<0.1 μm) to PM10. The geography and meteorological conditions in IV expose the community to a unique combination of natural and man-made pollutants. These sources include agriculture, industrial plants, and large cities across the border of Mexico, as well as the Salton Sea. To account for seasonal changes and source variation, our sampler collects particles from different wind directions over the course of a year. Each PM sample is chemically characterized and screened in an in vitro system to before moving into an in vivo model of asthma. Due to the high incidence of asthma in youth in IV, we are specifically interested in how this PM may modulate sensitization of the immune system to house dust mite (HDM) allergen, and in turn, how this impacts subsequent encounters with the allergen. Our initial chemical characterization indicates organic species dominate IV particles (58%) followed by ammonium sulfate (37%). The chemical composition of the organic particles from IV is highly complex and likely composed of hundreds of carbon-containing compounds. An in vitro screening in a human macrophage cell line demonstrates a significant increase in gene expression of COX-2, CYP1a1, IL-8 and IL-1B cytokines, as well as a similarity between IV PM and other agricultural regions in California. The high average degree of variability in the high organic nitrogen content suggest that ultrafine PM in IV is likely toxic and could be a major culprit for the health problems in the region. The increase of inflammatory cytokines when exposed to IV PM in vitro suggests the need for further testing in a murine model of asthma.
The Effects of Inhalation Exposure to Traffic-Generated Air Pollutants on Angiotensin II Receptor Expression and Signaling of Monocytes/Macrophages in the Vasculature and Kidney of Wildtype Mice on a High-Fat vs. Low-Fat Diet

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While multiple studies have reported a positive correlation between exposure to traffic-generated air pollution and exacerbation of cardiovascular disease (CVD), and more recently kidney diseases, very few studies have focused on the effects of these exposures in a healthy animal model. Furthermore, little is known on whether these exposure-related effects are exacerbated by concurrent consumption of a high fat diet. The renin-angiotensin system (RAS), when dysregulated, is known to mediate pathogenesis in the renal and cardiovascular system through Angiotensin (Ang) II signaling via the Ang II Type 1 (AT1) and/or Type 2 (AT2) receptors. We have previously reported that plasma Ang II levels are increased in C57Bl/6 wildtype mice exposed to traffic-generated pollutants. Thus, we hypothesized that inhalation exposure to traffic-generated pollutants results in altered inflammation in the vasculature and/or kidneys. To test this hypothesis, 3 mo old male C57Bl/6 mice on either a high-fat “Western” diet (HF, 21% fat) or standard (LF, low fat) mouse chow were randomly assigned to inhalational exposure of either filtered-air (FA n=10 per diet) or a mixture of 70 µg PM2.5/m3 diesel exhaust + 30 µg PM1/m3 gasoline exhaust (MVE: n=10 per diet) for 6 hr/day for 30 days. MVE exposure resulted in increased expression of the AT1 receptor in the vasculature, as determined by RT-qPCR and immunofluorescence, which was further exacerbated in MVE+HF exposed mice. In contrast, CYP1A1 mRNA levels were decreased with either HF diet or MVE-exposure, compared to FA+LF. No statistical change was observed in renal AT-2 levels across groups. Such findings indicate that inhalation exposure to traffic-generated pollutants can promote induction of RAS signaling, associated with factors that mediate the initiation and progression of vascular and kidney disease. Funded by NIH R15ES026795 (AKL) and an UNT Undergraduate Research Grant (BP).

Traffic-Generated Air Pollution-Mediated Alterations in Cerebral AhR and CYP Enzyme Expression Dependent upon Age and Diet in C57Bl/6 Wild Type Mice

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Inhalation exposure to traffic-generated air pollutants have been reported to have deleterious effects on the central nervous system (CNS), including blood brain barrier (BBB) disruption and neuroinflammation, 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 substances. Altered expression of certain CYP enzymes are associated with neurodegenerative disorders, thus we chose to investigate the effects of inhaled traffic-generated air pollutants on expression of these enzymes in the CNS on the BBB. Additionally, we investigated the expression through speculative and experimental regulation of cerebral hydrocarbons and AhR regulates transcription of CYP1A1 and 1B1, we analyzed its expression in the cerebrum and microvasculature, as well. Young (2 mo) or aged (18 mo) male C57Bl/6 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 AhR and 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 AhR and 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 C57Bl/6 mice on a low fat diet. There was no significant change observed in expression of CYP1A1 in either young or old mice. In the high-fat fed C57Bl/6 mice, MVE-exposure resulted in 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 altered cerebral expression of AhR and CYP enzymes in the young vs. aged differently (typically inversely), that is further complicated with concurrent consumption of a high fat diet. Research funded by NIEHS R00ES0126586 (AKL).

Angiotensin II Receptor Type 1 (AT1) Mediates Alterations in Blood Brain Barrier Integrity and Inflammation in Wildtype Mice, on Either a High- or Low-Fat Diet, Exposed by Inhalation to Vehicle Emissions

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Exposure to traffic-generated air pollutants has been associated with detrimental outcomes in the central nervous system (CNS), including exacerbation of neuroinflammation, neurodegeneration, and cerebrovascular disorders (stroke). We have previously reported that inhalation exposure to mixed vehicle exhaust (MVE) results in altered blood brain barrier (BBB) integrity and permeability in C57Bl/6 mice. The purpose of this study was to investigate whether Angiotensin II receptor Type 1 (AT1) signaling mediates alterations in BBB integrity, related to MVE-exposure, as well as whether concurrent consumption of a high fat (HF) diet exacerbates such outcomes. To investigate the aims of this study, we utilized both in vivo and in vitro (BBB co-culture) methodology. 3 mo-old male C57Bl/6 mice on either an HF or low fat (LF) diet were randomly assigned to inhalational exposure of either filtered-air (FA) or 30 µg PM/m3 gasoline exhaust + 70 µg PM/m3 diesel exhaust (MVE) for 6 hr/day for 30 d. Exposure to MVE + HF diet resulted in a significant increase in plasma Ang II, associated with increased BBB permeability (Na-F transport into the CNS), decreased tight junction (TJ) protein expression and increased expression of AT1 in the cerebral microvasculature. BBB co-cultures treated with plasma from mice in the exposure study showed significantly increased transendothelial electrical resistance (TEER), a measure of membrane integrity, and reduced TJ protein expression in the MVE+HF group, compared to FA+LF or FA+HF groups. However, when pre-incubated with the AT-1 inhibitor, Losartan, TEER and TJ protein expression in BBB co-cultures treated with plasma from mice were not statistically altered in MVE+HF vs. control groups. Both interleukin-6 and transforming growth factor-β were also observed to be statistically increased in astrocytic media from BBB co-cultures treated with plasma from MVE+HF groups, compared to plasma from control animals. Our results indicate that inhalation exposure to traffic-generated air pollutants, coupled with an HF diet, results in altered BBB integrity, mediated through Ang II-AT1 signaling, and increased CNS inflammation. Funded by NIH R15ES026795 to AKL.

One Year Urban Nanoparticle Concentration Monitoring in Queens, New York

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Particulate matter in air is regulated in the United States by the Clean Air Act of 1970 and subsequent amendments. Standards exist for PM10 and PM2.5 particulate concentrations. These standards are supplemented by regulations and controls for other gaseous pollutants. However, as of this time, no regulations exist for airborne nano-particle concentrations such as those from mobile combustion sources, i.e. motor vehicle emissions. Further, other than black carbon measurement devices, few instruments exist that can document the prevalence of nano-particles in the global environment. To help diminish this environmental data gap, several instruments have been developed to measure urban as well as indoor nano-particle pollution. These can document the prevalence of nano-particles in the global environment, further, those from mobile combustion sources, i.e. motor vehicle emissions. Additional to the above, recent regulations and controls for other gaseous pollutants. However, as of this time, no regulations exist for airborne nano-particle concentrations such as those from mobile combustion sources, i.e. motor vehicle emissions. Further, other than black carbon measurement devices, few instruments exist that can document the prevalence of nano-particles in the global environment. To help diminish this environmental data gap, several instruments have been developed to measure urban as well as indoor nano-particle pollution. These are measurements most often made using passive charging of particulates in the under 10 to 300 or more nanometer size range. From the charge measurements, particulate surface area estimates may be calculated and expressed as Lung Deposited Surface Area (LDSA) from urban and residential atmospheres. Particulates in this size range are out of the usual optical and gravimetric measurement range. Two devices, the Naneos Partector and the Pegasor Urban air monitor are commercially available. To document LDSA in urban air, three Pegasor Urban units were co-located at the New York State DEC monitoring station at Queens College, Queens, New York. The units would be operated in an unattended manner for one full year. As of July, 2018, after 9 months of operation and using a proprietary algorithm to estimate PM2.5, the units agreed with each other within ±20% for 95% of the time. This level of agreement between the 3 units had an R2 value of 0.64 to 0.66. Agreement between the 3 units had an R2 value of 0.64 to 0.66. Data analysis continues until the end of the 2018 year since other co-located gravimetric instruments report their data with delays of up to 90 days. In comparison to the TEDM...
and gravimetric instruments requiring daily or weekly attendance, the passive charge devices employed here, once located in the field and supplied with an internet or cell phone connection, required no maintenance, no human intervention and reported their observations to the cloud. This allowed minute display of urban air LDSA concentrations with controlled or open global access. Additional data analysis as well as other comparisons are ongoing and will be reported. This work was self funded and supported by Pegaso Oy, who supplied the measuring devices. The cooperation and hospitality of the staff of the NY DEC is gratefully acknowledged.

1089 Developmental Traffic-Related Air Pollution Exposure and Autism-Related Neurotoxicity in Mice
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Epidemiological findings have suggested that developmental exposure to traffic-related air pollution (TRAP) may be associated with an increased risk of autism spectrum disorders (ASD). In order to further assess this association mechanistically, we carried out a series of studies in C57Bl/6J mice developmentally (EO to PND21) exposed to 250-300 µg/m3 of diesel exhaust (DE) or filtered air (FA) in a controlled environment. A series of behavioral, molecular, and brain histological assessments relevant to ASD were carried out in both male and female pups and young adults. Behavioral testing indicated changes consistent with ASD, namely increased repetitive behavior, disrupted social behavior and impaired social communication. Elevated levels of the inflammatory cytokine interleukin-6 (IL-6) were found in fetal brains and placenta of mice exposed to DE. Prompted by findings obtained in the maternal immune activation model of ASD, we also explored a possible biochemical pathway relevant for ASD which may be disrupted by developmental DE exposure. Additionally, increased levels of phosphorylation of STAT3, as well as increased expression levels of DNMT1 (DNA methyltransferase 1) and reelin (RELN) were found to be associated with DE exposure. DNMT1 is a target gene of activated transcription factor STAT3 and has been shown to bind directly to the promoter region of the RELN gene via epigenetic modification. The RELN gene encodes for extracellular protein reelin, which plays a major role in guiding the neuronal migration process during CNS development. Downregulation of RELN due to DE exposure persisted in cortical samples of PND60 male mice. An immunohistochemical analysis was performed with the lamina-specific markers RELN and calretinin to examine the cortical organization in PND60 brains of mice exposed to either DE or FA during development. Significant differences were seen in the cortical distribution of both RELN- and calretinin-positive cells, suggesting a disruption of cortical layering. Altogether these studies suggest that developmental exposure to DE causes behavioral, biochemical and histological changes in mice which are similar to those observed in ASD. Supported in part by NIEHS grants RO1ES22949, and RO1ES028273.

1090 Mouse Pulmonary Response Induced by Exposure to Dust from Sawing Corian, a Solid-Surface Composite Material
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Corian®, a solid-surface composite (SSC), is composed of a proprietary blend of powdered alumina trihydrate, methyl methacrylate, and other binders and colorants. The present study evaluated the murine pulmonary toxicity from exposure to dust generated from sawing of SSC. The particle number-based geometric mean diameter and geometric standard deviation $\sigma$ of the airborne fraction SSC sawing dust following suspension in saline was 1.16 µm, $\sigma = 2.18$, which is similar to the number-based geometric mean aerodynamic diameter to aerosolized SSC sawing dust of 1.05 µm, $\sigma = 1.78$. Inductively coupled plasma and energy dispersive X-ray spectroscopy analyses confirmed that aluminum was the principle inorganic component of SSC sawing dust particles as well as microbiome and chemical composition of the dust, with different doses (25, 50 and 100 µg/ml) of farm dusts. Toxicological and immunomodulatory potential of the dusts was measured. Dusts were also collected from farm 1 differed more significantly from each other than matrices. The results were dependent of the farm, as responses from samples collected from farm 2, were more significantly different from each other than matrices. The adsorption of proteins reelin in alveoli at all doses at day 1 post-exposure, and fibrosis in the 1000 µg dose at day 14 post-exposure. Darkfield imaging indicated alveolar cell deposition and granulomatous mass formation persisting to 14 days post-exposure in all exposure groups. Taken together, these findings suggest that SSC sawing dust exposure may induce pulmonary inflammation and damage that warrants further investigation.

1091 Effect of Vinyl Chloride on HNF4α Expression in a Model of Nonalcoholic Steatohepatitis (NASH)
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Vinyl Chloride (VC) is an organochlorine compound which is known to cause hepatic steatosis, fibrosis, necrosis and hepatocellular carcinoma. Occupational exposure has been directly associated with hepatic angiosarcoma. Recent studies have shown that inhalation exposure to VC at levels below current Occupational Safety and Health Administration exacerbates liver injury, steatosis and inflammation in mice fed a high fat diet suggesting that VC is a Toxicant Associated Steatohepatitis (TASH) causing agent. Previous studies from our laboratory have shown that decrease in expression and activity of Hepatocyte Nuclear Factor 4 alpha (HNF4α) an orphan nuclear receptor produces NASH like pathology in the liver. We hypothesized that VC might be causing these effects by targeting HNF4α. To test this hypothesis, HNF4α promoter/mRNA and protein expression and activity was measured in livers from mice with and without inhalation exposure to vinyl chloride and fed either normal chow (LFD) or high fat diet (HFD). Western Blot and RT-PCR analyses were used to determine HNF4α protein expression and activity. Western blot analysis for adult isoforms (P1) of HNF4α showed no difference in protein expression between treated and untreated LFD fed animals. Interestingly, the adult P1 HNF4α protein levels were decreased in untreated HFD fed mice but returned to normal levels with VC treatment. Expression of fetal P2 HNF4α isoforms were not affected by VC treatment but were induced in all mice fed HFD. RT-PCR analysis of HNF4α and its target genes did not reveal any changes in HNF4α activity measured using target gene expression except CD36, a lipid importer, which significantly increased in mice on a high fat diet. VC treatment decreased CD36 expression in mice on HFD, but expression was still significantly greater than VC treated and untreated fed LFD. In conclusion, HFD decreased HNF4α expression and VC exposure along with HFD prevented this decrease. Further, the HFD treatment with and without VC induced expression of the fetal P2 isoform in HFD. Further studies are required to determine the mechanisms behind these observed effects.

1092 The Effect of Farm Dust Collection Method on the Toxicological Responses in Lung Co-Culture Model
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Early life exposure to farm environment has been shown to protect from asthma and allergies, whereas exposure during adulthood can increase the risk of respiratory syndromes. To assess the immunological mechanisms behind these phenomena, the focus of research has shifted to in vitro stimulations with farm dust. However, the use of authentic farm dust raises questions about the collection methods as different methods might affect the composition of the dust and furthermore alter toxicological and immunological responses. Detailed characterization, such as data on the size distribution of dust particles as well as microbiome and chemical composition of the dust, could also support a better identification of cause effect relationships. The aim of our study was to assess the impact of dust collection methods on cellular responses. Farm dust samples were collected from 3 different Finnish dairy farms by 1) mechanical scraping (sample matrix 1), 2) settled dust sampling (sample matrix 2), and 3) utilizing DGI impactor (sample matrix 3). Scraped sample was further filtered into two size-fractions (63-125 µm). DGI filter samples were extracted and different size fractions were pooled to obtain sufficient mass of particles for the experiments. Co-cultures of lung epithelial cells (A549) and macrophage-like cells (THP-1) were stimulated with different doses (25, 50 and 100 µg/ml) of farm dusts. Toxicological and immunomodulatory potential of the dust was measured. Dusts were also analysed for particle size distribution, microbiome and chemical composition. Cell membrane integrity was not affected by the collection method, whereas oxidative stress and metabolic activity responses were distinct between matrices. The results were dependent of the farm, as responses from samples collected from farm 1 differed significantly from each other than matrices from other farms. The secretions of IL-6 and TNFa were slightly dependent on matrix. The response profiles were similar whereas the absolute levels
were slightly lower in matrix 2 and higher in matrix 3. Our results indicate that the method of collection slightly affects the toxicological and immunological responses induced by farm dust. This should be taken into account when interpreting the results and comparing findings of different studies as well as when searching for causative components.

1093 Nanjing Youth Olympic Games 2014 Air Quality Intervention: Changes in the Chemical Composition and Toxicological Characteristics of Size-Segregated Urban Air Particulate Matter

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The global health risk presented by ambient air particulate matter (PM) calls for efficient emission reduction strategies, especially in areas with dense population and high PM concentrations, such as Chinese megacities with high traffic and industrial activity. In August 2014, Nanjing, a megacity of over 8 million inhabitants in Eastern China, held the Youth Olympic Games (YOG). To provide cleaner air for the event, Nanjing and several surrounding cities enacted temporary emission control measures, including shutting down industries and construction sites, and limiting traffic. In this study, our aim was to assess how this air quality intervention affected the chemical composition of PM and the induced cytotoxic and oxidative stress responses in a co-culture model of alveolar epithelial cells and macrophages. We collected day- and nighttime size-segregated urban air PM in Nanjing before (June), during (August), and after (October) the YOG emission control period, followed by extensive chemical characterization. Co-cultures of A549 cells and THP-1 cells differentiated into macrophage-like cells were exposed to three doses (SO2, 100, 200 μg/mL) of PM in each size range (PM0.25, 0.25–1.0 μm, PM1.0, 1.0–2.5 μm, PM2.5, 2.5–10 μm). After 24 hours, cellular metabolic activity (CMA), cell membrane integrity, intracellular oxidative stress, and thiold redox state were measured. In general, PM0.25 elicited high oxidative stress response and a moderate reduction of CMA, yet showed little to no changes in cell viability and generally modest thiold redox state changes. For the three smaller size ranges, the role of oxidative stress diminished compared to PM2.5, while the thiold redox status and CMA showed similar or greater detrimental effects compared to PM0.25. For all end-points, the August samples showed lower detrimental effects compared to June samples, while the effects returned to higher levels for October samples. In conclusion, we observed changes in chemical composition of PM due to the emission control measures in August, which were reflected as less severe responses in many toxicological endpoints. Thus, air quality intervention not only reduced the ambient concentration of airborne PM, but also decreased its toxicity. However, some of the greatest toxicological responses were seen for October PM, indicating loss of beneficial effects of the temporary emission control period.

1094 Assessment of Cured-in-Place Pipe Worksite Emissions and Toxicity

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The U.S. water infrastructure is in need of widespread repair due to age-related deterioration. Currently, the cured-in-place pipe (CIPP) procedure is the most common and cost effective method for water pipe repair. This method involves the on-site manufacture of a new polymer composite plastic pipe within the damaged pipe. This curing process, however, results in the release of emissions into the environment. Our recent investigation of public records has determined public health incidences near CIPP installation sites in at least 29 states resulting building evacuations and health agency responses. Field investigations at 5 CIPP installation sites revealed transient chemical plumes characterized to contain a variety of compounds (styrene, acetylene, toluene, benzaldehyde, phenol, etc.) at differing concentrations. To understand hazards associated with exposure, an in vitro toxicity assessment was performed utilizing mouse lung epithelial and macrophage cell lines and emission samples collected at 4 worksites. All samples were normalized based on the major particulate species, and CMA, a marker that corresponds with lung disease severity, was determined to be up-regulated in all cells exposed to CIPP emissions but down-regulated in styrene controls. Further, vascular cellular adhesion molecule-1, a marker of inflammation, was induced following all exposures except for site 3, which was determined as the least cytotoxic. The immune mediator, transforming growth factor-β and heme oxygenase-1, a marker of oxidative stress, were elevated only after CIPP emission exposures but not by styrene alone. Together our findings demonstrate risks associated with exposure to the CIPP process and the need for worksite interventions in regards to emissions and toxicity. This suggests the need for adjustments in operational procedures to reduce potential public adverse health effects.

1095 Co-exposure of Air Pollutants in Human Lung In Vitro Models


Background Long-term exposure to air pollution including diesel exhaust particles (DEP), nitrogen oxides (mainly NO2), and sulfur dioxide (SO2) has been associated with adverse effects to human health. Most studies are epidemiologically based and often lack methodologies to establish exposure-effect relationships. Aim To develop new exposure systems to allow for repeated co-exposures of gases (NO2, SO2, and DEP) with human bronchial mucosa in vitro lung models at the air-liquid interface (ALI) to assess inflammatory responses that are important for respiratory disease development. Method Bronchial mucosa models were developed using human primary bronchial epithelial cells (PBEC) cultured at ALI and co-cultured with or without macrophages (PBEC-ALI/MQ and PBEC-ALI, respectively). Single and repeated gas exposures (low: 0.1 ppm NO2, 0.2 ppm SO2; high: 0.2 ppm NO2, 0.4 ppm SO2) with and without DEP (12.5 μg/cm2) were carried out at, including clean air (sham) exposure. Changes in gene expression and protein levels were measured as markers for pro-inflammatory (CXCL8) and tissue injury (MMP9) responses. Cell viability was assessed using the lactate dehydrogenase assay. Results More than 90% of the cells were viable before and post repeated and combined exposure to gases and DEP. Sham exposure significantly increased release of CXCL8 in PBEC-ALI/MQ compared to in PBEC-ALI after 24h post exposure. High NO2 and SO2 exposure significantly increased release of CXCL8 in PBEC-ALI. However, 48h repeated exposure to both low and high concentration of both gases resulted in reduced release of CXCL8 in PBEC-ALI compared to sham. Similarly, release of CXCL8 was also significantly reduced in PBEC-ALI/MQ at both 24h and 48h post-repeated exposure to gases. Further, significantly increased release of MMP9 (tissue injury marker) was detected in PBEC-ALI after combined exposure to DEP with gases. Conclusion Combining the newly developed exposure system, which allows for repeated and combined exposure of gases and diesel exhaust, with the physiologically relevant multicellular in vitro lung models, pro-inflammatory and tissue injury responses could be investigated. This approach would be useful to further investigate the underlying mechanistic toxicity aspects of known air pollution components.

1096 Evaluation of Tire and Road Wear Particles in Air in Delhi, India


Tire and road wear particles (TRWP) are formed at the frictional interface of the tire and the road surface, and include contributions from both the tire and the pavement. In a previous international sampling campaign to quantify TRWP in environmental media, TRWP were found to be ubiquitously present in ambient air, detectable in both the PM10 (particulate matter less than 10 μm) and PM2.5 (particulate matter less than 2.5 μm) fractions; although at low concentrations relative to other PM in the air. However, previous sampling was executed in developed countries, where ambient particulate matter concentrations are well-managed. The purpose of this study was to understand the TRWP concentrations and contributions to ambient particulate matter in a developing country with known challenges associated with ambient air particulate. As a city with one of the highest concentrations of ambient PM, Delhi, India was selected for investigation in this study. Both PM10 and PM2.5 air samples were collected over consecutive days at six locations throughout Delhi. Total suspended particulate (TSP) samples were also collected at two of the six locations. Study sites were selected to represent a wide variety of human receptors with close proximity to a traffic source; samples were collected within 10 m of the roadside. These air samples were then evaluated for the presence of TRWP using a previously-established pyrolysis gas-chromatography-mass spectrometry method, which measures pyrolys products of the rubber polymer to ensure specificity to TRWP. Mean TRWP air concentrations across the six sampling sites were 0.45, 2.1, and 6.2 μg/m3 for PM2.5, PM10, and TSP, respectively.
respectively. These concentrations represented 0.40%, 0.58%, and 0.50% of total particulate in these respective size ranges, indicating that TRWP is only a minor contributor to ambient particulate matter in Delhi. This finding is consistent with sampling in developed countries, where TRWP represented less than 0.3% of total PM2.5 and less than 0.6% of total PM10 on average. That said, the concentration of TRWP in Delhi was higher than found elsewhere; though the underlying reasons for this are unknown, differences in traffic volume, road surfaces, and tires may be potential contributors.

### 1097 In Vivo Toxicity Assessment of Metal Contaminated Wind Blown Particulate Matter from an Abandoned Uranium Mine on the Navajo Reservation

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Studies have shown that particulate matter (PM) adversely affects the respiratory and cardiovascular systems, and that specific metals are important modifiers of PM toxicity. We recently found that PM derived from the sediments surrounding an abandoned uranium mine (AUM; Claim 28) in northern Arizona showed to be more toxic than PM from non-impaired sites. Here, we address the extent to which uranium-contaminated PM is present in ambient air of the community and further assess cardiopulmonary toxicity in acute and subchronic exposures. C57BL/6 and ApoE−/− (a model of vascular inflammation) mice were exposed to concentrated ambient PM (~80 µg/m3) in the Mobile Air Research Lab for 1 or 28 days for 4 hours/day. The mobile laboratory was located 1 km SW of the AUM. Wind stations were positioned on the mobile laboratory and on the AUM to determine wind directions and speed. Lungs and aortas were obtained and analyzed for inflammatory disease marker transcripts (TNF-β, TGF-β, CXCL1, and IL6). Bronchoalveolar lavage was conducted to examine influx of macrophages and neutrophils into the lungs. QPCR for lung 1-day exposure showed an increase in TNF-α, IL6, and TGF-β in PM groups versus filtered air (FA)-exposed mice, and CXCL1 levels slightly decreased as seen in both 1- and 28-day mice. In the 28-day exposures, CXCL1, TGF-β, and TGF-β levels were lower in PM mice than FA mice. QPCR for aorta 1-day exposure showed a slight decrease in CXCL1 in PM mice compared to FA mice, and a small increase in IL6. In the 21-day exposures, slight increases were seen in all probes of PM mice compared to FA mice. Average wind direction over the mobile laboratory traveled from SW to NE, and mostly north over the AUM, away from the mobile laboratory and community. Interim results show minimal inflammatory or toxic outcomes from concentrated ambient PM in the region adjacent to the AUM, but also minimal metals contamination, largely due to predominant wind flow. The next step will be to fully assess the metal content of the PM by ICP-MS and associate with daily wind direction patterns.

### 1098 Evaluating Toxicity of Inhalation Exposure to Unconventional Natural Gas Drilling-Related Chemicals

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Over the past two decades, an effort towards reducing reliance on imported fossil fuels has resulted in the rapid expansion of natural gas drilling in the United States. Shale gas made up 40% of U.S. gas production in 2012, compared to 2% in 2000, and is projected to grow fourfold by 2035. The rapid expansion of unconventional natural gas drilling (UNGD) has resulted in drilling moving closer to rural and urban communities, however the chemical composition and toxicological relevance of UNGD-related chemical mixtures is largely unknown. Studies show that residents living less than 0.5 miles from an UNGD site are at higher risk and have higher exposure to UNGD-related chemicals than individuals living more than 0.5 miles from an UNGD site. Air, water, and personal passive sampling devices around the respiratory zone of mares, serving as a sentinel for humans, were deployed at two farm locations with the same proprietor. The affected farm in Pennsylvania had an UNGD site 0.25 miles from its location and another five within a 2.5 mile radius, the New York location served as a control site with no UNGD sites in its vicinity. Over the 18 month sampling period, approximately 56% of full-term neonatal foals born at the Pennsylvania location were dysphagic (difficulty swallowing), compared to 12% at the New York location. These findings suggest that UNGD-related chemical exposure may be associated with the adverse health outcome seen in equine and should be explored for the potential toxicity in a human inhalation model. Quantification of polycyclic aromatic hydrocarbons (PAHs) from passive samplers identified dibenz(ghi)chrysene, phenanthrene, and benzo[a]pyrene, at higher concentrations in PA compared to NY. Exposure to these individual PAHs (1-500 µg/ml) in a 3D human bronchial epithelial cell (HBEc) culture model resulted in significant (q<0.05) induction of transcriptional biomarkers associated with oxidative stress, DNA damage, barrier function and xenobiotic metabolism indicating potential mechanisms for toxicity. Surrogate mixtures identified from a classification approach used to identify chemical exposure profiles associated with location will be tested in the HBEc and assessed to identify the potential adverse effects associated with inhalation exposure to UNGD-related mixtures.

### 1099 Genetic Injury Caused by LINE-1 Retrotransposition following Intratracheal Instillation of Benzo(a)pyrene in ORFeus1,2 Transgenic Mice

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Long interspersed nuclear elements (LINE-1) are a group of non-LTR-retrotransposons in the human genome. LINE-1 is involved in both disease initiation and progression via retrotransposition dependent and independent mechanisms. Benzo(a)pyrene (BaP) is a widespread polycyclic aromatic hydrocarbon known to be mutagenic and to alter the lung epigenome via mechanisms linked to LINE-1 retrotransposition. These alterations may contribute to heightened rates of lung cancers; cancer types often diagnosed in late stages and displaying the highest cancer mortality rate in the United States. As such, there is need to identify potential biomarkers relevant to the diagnosis and management of patients with lung cancer. The experiments described here focused on the study of genetic injury caused by LINE-1 retrotransposition in ORFeus1,2 mice treated with the lung carcinogen BaP. Eight groups of ORFeus mice, including four male and four female mouse groups were examined. The carcinogen was administered via intratracheal instillation with or without CRE recombinase for conditional activation of a single-copy LINE-1 transgene in the mouse lung. Significantly higher expression of ORFeus LINE-1 mRNA was observed in both male and female lung glands combined and BaP treatment compared to either BaP alone or vehicle. Interestingly, the lung carcinogen alone was capable of inducing LINE-1 mRNA expression in the lung of treated mice. LINE-1 expression was higher in female lungs compared with males, suggesting that females are more susceptible to epigenetic disturbances by BaP. Furthermore, RT-PCR analysis of eight genetic targets within an in silico predicted LINE-1 regulatory network showed increased expression, establishing LINE-1 as a key regulator of genome integrity in the murine lung. These findings are now being used for testing precision-based therapies that can neutralize the genetic injury caused by toxic environmental exposures in vivo that activate LINE-1. We conclude that the ORFeus mouse is a sensitive model to evaluate complex biological interactions that occur during the course of chemical lung carcinogenesis in vivo.

### 1100 Investigating the Role of TRPV3 in Lung Epithelial Cell Repair

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Transient receptor potential vanilloid-3 (TRPV3) is a Ca2+ ion channel. In the skin, TRPV3 regulates keratinocyte proliferation and cornification during wound repair. Our laboratory recently characterized the expression of TRPV3 in human lung epithelial cells, and its contributions to the pneumotoxic effects of wood smoke particulate matter (PM). Over-expression of TRPV3 in primary human bronchial epithelial cells sensitized these cells to cytotoxicity by wood smoke PM, which was partially blocked by a TRPV3 antagonist. Additionally, mice treated sub-acutely with pine PM via oropharyngeal delivery exhibited increased airway resistance that was blocked by TRPV3 inhibition. In mice, histological changes in the major airways, consistent with active wound repair, were observed and was TRPV3-dependent. Transcriptomic profiling of BEAS-2B and BEAS-2B TRPV3-overexpressing cells with and without wood smoke PM or agonist treatment demonstrated significant differences in the regulation of EMT-specific genes including N-cadherin, collagen I, the structural protein vimentin, and numerous genes in the EGFR network. Notably, we have found that TRPV3 overexpression as well as TRPV3 agonists and antagonists appear to “lock” cells into a non-migratory epithelial phenotype, in part through an apparent attenuation of EGF activation following epithelial cell/membrane damage. These data suggest that TRPV3 activation not only contributes to the acute cytotoxic effects of wood smoke PM to lung epithelial cells but also plays a role in coordinating signaling events involved in epithelial wound repair following damage. The mechanism by which TRPV3...
regulates lung epithelial wound repair is unknown, but the consequences of stimulation or inhibition of this process by TRPV3 agonists and antagonists has both toxicological and therapeutic significance.

### 1101 Apoptosis Resistance of Fibroblasts Precedes Progressive Scarring in Pulmonary Fibrosis and Is Partially Mediated by Toll-Like Receptor 4 Activation

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Inhalation of environmental toxicants such as cigarette smoke, metal or wood dust, silica, or asbestos is associated with increased risk for idiopathic pulmonary fibrosis (IPF). IPF involves progressive scarring of lung tissue, which interferes with normal respiration and is ultimately fatal. To develop therapies to reverse or prevent IPF, it is crucial to clarify the complex cellular mechanisms of IPF pathogenesis. Fibroblast apoptosis is essential in normal wound healing but is dysregulated in IPF. Recent studies suggest that Toll-like receptor 4 (TLR4) is key in the onset of IPF. Mutations in TOLLIP (TLR4 inhibitor) are associated with increased risk for IPF, and TLR4-defective or -deficient mice are fibrosis resistant. Moreover, TLR4 activation causes pro-survival signaling, potentially helping cells evade apoptosis. We hypothesized that epithelial damage leads to the endogenous TLR4 agonist HMGB1, which causes fibroblast apoptosis resistance and thus interferes with wound resolution, contributing to IPF development. Radiation-induced PF was used as a model for IPF because it very closely mimics the progressive and intractable nature of IPF. Female C57BL/6J (WT) and C57BL6/J B6.10S0CN-Tlr4<sup>del/del</sup> (TLR4KO) mice were exposed to 13 Gy whole-thorax ionizing radiation (rt), a fibrosis-inducing dose in WT mice. At 24 hr, 8 wk, and 22 wk post-irradiation (PI), samples collected included plasma, right lungs for primary mouse lung fibroblast (1<sup>st</sup> MLF) expansion, and left lungs for histology. Plasma samples showed increased HMGB1 by ELISA during the inflammatory phase of rt response at 8 and 12 wk PI in both WT and TLR4KO strains. However, TLR4KO lungs exhibited less fibrosis than WT lungs at 22 wk PI, assessed by modified Ashcroft scoring. Furthermore, apoptosis of 1<sup>st</sup> MLFs was induced and the robustness of the apoptotic response was quantified. It was determined that WT 1<sup>st</sup> MLFs were not apoptotic resistant at 24 hr; however, this phenotype was found as early as 8 wk PI and persisted at 12, 16, and 22 wk PI timepoints. These findings expand current knowledge of PIF by indicating that apoptosis resistance occurs earlier in the rt response than previously assumed. Furthermore, apoptosis resistance was not detected in TLR4KO 1<sup>st</sup> MLFs, suggesting that TLR4 plays a key role in fibroblasts acquiring this detrimental phenotype. Future studies will explore TLR4 pro-survival signaling in vitro to further characterize the mechanisms of pulmonary fibrosis.

### 1102 The Effect of Enriched vs. Inadequate Housing Conditions on Biomass Smoke-Induced Cardiovascular Dysfunction in Mice

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Air pollution increases the risk of cardiovascular (CV) morbidity and mortality, even with short term exposures. Extrinsic factors that cause psychosocial stress may increase susceptibility to the CV effects of air pollution. Lower socioeconomic status, lack of physical activity and green spaces (i.e. urbanization) is associated with deleterious CV changes. Noise, extreme temperatures, and inadequate social environment lead to stress and CV changes in rodents. In addition, wildfires increasingly threaten public health as they become more widespread and occur more frequently at urban/wildland interfaces. Biomass smoke is an understudied air pollutant. We hypothesized that socioeconomic status, lack of physical activity and green spaces (i.e. urbanization) may take place. To shed light on such interactions we used our established in vitro model of isolated trigeminal ganglion (TG) neurons of CD1 mice (PDNS) that predicts the potency of chemicals to interact with chemoreceptors by using Fura-2-AM based Ca<sup>2+</sup> imaging. To reduce the variety of VOCs we restricted our experiments to (a) prototypical emissions from oriented strand boards (OSB), (b) the five VOCs with the highest concentrations, and (c) one representative mixture of these five compounds. The TG-neurons were stimulated in ascending concentrations of OSB, of the single compounds (i.e. α-pinene, β-pinene, isopropyl-n-hexanal, and trans-2-octanal. Based on the percentage of responding neurons the EC<sub>50</sub> values were: α-pinene: 59.5 mM, β-pinene: 3.6 mM, limonene: 5.2 mM, hexanal: 1.9 mM, and trans-2-octanal: 0.3 mM. The five compounds were mixed in ratios derived from representative air sample taken in the test chambers at the Thünen Institute. Using the same ratios, the EC<sub>50</sub> values of the single compounds predicted a pure additive EC<sub>50</sub> of 6.8 mM for the mixture. The experimental evaluation of the synthetic mixture yielded an EC<sub>50</sub> of 4.9 mM. These results confirmed that the aldehydes are more potent in causing sensory irritation than the selected terpenes. Moreover, using in vitro assay no additive of a mixture representing emissions from OSB-boards could be detected. However, this novel approach as some limitations that need to be considered when extrapolating the results to indoor air exposures of humans.
1105 Baroreflex Sensitivity and Cardiovascular Responses to Acute Peat Smoke Inhalation in Rats


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Exposure to air pollution elicits disruptions to internal homeostatic controls, the abnormal functioning of which may increase risk for adverse cardiovascular events. The purpose of this study was to assess the concentration-dependent impacts of acute exposure to peat smoke (PS), a key wildland fire-related air pollution source, on cardiovascular function and sensitivity of the baroreflex (BRS), which maintains homeostatic control of blood pressure. Three-month-old male Wistar-Kyoto rats implanted with radio-telemeters to monitor heart rate (HR), blood pressure (BP), and the electrocardiogram, were exposed once, for 1 hr, to filtered air (FA) or low (LP: 0.36 mg/m³ fine particulate matter (PM)) or high (HP: 3.76 mg/m³ PM) concentrations of smoldering PS, generated using a tube furnace system. Spontaneous baroreceptor sensitivity was assessed via the sequence method, which analyzed sequences of fluctuations and corresponding changes in pulse interval using custom software. Exposure to HP, but not LP, caused increases in systolic (11.6%; p=0.053) and diastolic BP (15.6%; p=0.02) and a decrease in HR (p=0.065) during exposure relative to FA. At hour 2 after exposure, HP caused a significant positive linear trend in systolic BP (p=0.059) and a significant increase in diastolic BP relative to both FA and LP. For the first 3 hours after exposure, HP caused an increase in diastolic BP relative to FA and LP (both with p<0.05). By contrast, only exposure to LP increased BRS (i.e. increased gain of all sequences) relative to FA during exposure (LP: p=0.052, FA: p=0.87), previously determined to increase sensitivity to triggered cardiac arrhythmias. Interestingly, PM size (LP = 0.7 microns vs. HP = ~1.3 microns) and proportion of organic carbon (LP = 77% vs. HP = 67%) varied with exposure level. In summary, exposure to air pollution may cause homeostatic shifts, the exact consequences of which are not known, but may contribute to increased cardiovascular risk.

1106 Multiple Comparison of Combustion Emission Toxicity of Wood, Diesel and Aged Equivalents on Novel Thermophoretic Air-Liquid Interface Exposure System


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In attempt to lower fossil fuel usage, the European Union has encouraged among other renewable sources, wood to be used as a heating source. However, biomass combustion increases particulate matter (PM) levels in many regions and heating has become one of the biggest sources of PM especially in winter time. Effects of wood combustion and especially in aged aerosols on health are still largely unknown even many diseases have been connected to PM exposure. We measured several different smoke emissions with and without aging. Used aging conditions were created using LP = 77% vs. HP = 67%) which not only may contribute to increased cardiovascular risk. Moreover, the precise reason for the divergent BRS and BP responses among exposure levels is unclear, but may relate to physicochemical factors specific to each exposure level. Disclaimer: This abstract does not reflect US EPA policy.

1107 Inflammatory Responses in Mouse BALF after Exposure to Fresh or Aged Spruce or Lignite Combustion Aerosol


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Lignite and wood smoke produced due to residential heating are sources of fine particulate matter (PM), which have a consistent effect on human disease, for example exacerbation of cardiovascular diseases and respiratory diseases. The purpose of this study was to model the subchronic effect of wood and lignite smoke exposure on pre-challenged mouse lung. In this study, LPS instilled or healthy C57BL/6J mice (8-9 weeks old) (n=6) were exposed to combustion aerosol in a whole body inhalation chamber for 4 hours for three consecutive days. Four different aerosols were produced in a light stove: wood smoke (spruce), flow tube aged wood smoke (spruce), lignite smoke, or flow tube aged lignite smoke. The control group for both LPS mice (n=11) and healthy mice (n=12) were kept in the animal room separate from the exposure room throughout the experiment, and they were used as controls for all four exposure groups. From collected bronchoalveolar lavage fluid (BALF) total cell count, cell differential count, cell assay, and cytokine analysis (IFNγ, IL-10, IL-12p70, IL-1b, IL-2, IL-4, IL-5, IL-6, TNFα, and KC) were made. An increase can be seen in BALF cell number of LPS mice in contrast to the healthy mice in all aerosol exposed groups and in the control group. Macrophage number seems slightly higher in LPS treated groups, whereas lymphocyte and neutrophil number seems slightly higher in LPS treated groups. Were seen inside alveolar macrophages in BALF samples of fresh wood smoke and aged wood smoke exposed mice and, in less quantity, fresh lignite and aged lignite smoke exposed mice. Genotoxicity in BALF cells shows slight variation between different exposure groups and there is some differences between LPS instilled and healthy mice as well. All ten cytokines measured (IFNγ, IL-10, IL-12p70, IL-1b, IL-2, IL-4, IL-5, IL-6, TNFα, and KC) were low in concentration. Signs of neutrophil driven acute inflammation in LPS treated mice was expected since the LPS instillation induced inflammation is widely reported technique. Engulfed carbon agglomerates inside BALF alveolar macrophages, in both LPS and healthy mice, indicates the activation of inflammation immune response due to wood or lignite smoke exposure.

1108 Zebrafish Locomotor Responses Reveal That Irritant Effects of Biomass Smoke Are Influenced by Fuel Type, Burn Conditions, and Byproduct Chemistry


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Human exposures to wildfire-derived particulate matter (PM) have been linked to increased incidences of adverse heart and lung health outcomes. However, little is known about the influence of biomass fuel type and burn conditions on toxicity. The purpose of this study was to assess the irritant potential of extractable organic material (EOM) of PM derived from biomass smoke condensates from 5 fuels (Eucalyptus, Pine, Pine Needle, Peat, or Red Oak), representing various fire-prone regions of the USA, burned at 2 temperatures each (flaming at ~640 °C and smoldering at ~500 °C) in a locomotor assay in 6-day post-fertilization (dpf) zebrafish. We hypothesized that locomotor responses, measures of irritant effects, are dependent on fuel type and burn conditions and that these differences would relate to combustion byproduct chemistry. To test this, locomotor activity was tracked for 60 minutes in the dark in 6 dpf zebrafish embryos (28-32/group) exposed acutely to 0.4% DMSO vehicle or EOM from biomass smoke (0.3-0.3 µg EOM/ml half-log intervals from each of the 10 condensates in 96-well plates). All condensates elicited concentration-dependent responses. Linear regression analysis (of the first 20 minutes of concentration-response data) to derive rank order potency indicated that on a µg PM basis, flaming Pine and Eucalyptus demonstrated the greatest irritant effects. By contrast, on an emission-factor basis, which normalizes responses to the amount of PM produced/kg of fuel burned, smoldering smoke condensates showed a much greater capacity (~100-fold) to elicit irritant responses than flaming condensates, with smoldering Pine being the most potent. Importantly, irritant responses strongly correlated with polycy-
clic aromatic hydrocarbons (PAH) content (n=0.95, r²=0.90, p<0.0001), but not organic carbon or methoxyphenols. These results indicate that fuel type and burn conditions impact toxicity, and differences in responsiveness are likely determined by the quantity and chemical composition of PM. Furthermore, these results corroborate findings in mouse lung toxicity and PAH-sensitive Salmonella mutagenicity assays. Disclaimer: This abstract does not reflect US EPA policy.

1109 Health Effects of Wildfire Smoke Exposure

The recent wildfire season in the western United States has resulted in increasing levels of particulate matter (PM). This study examined the health impacts of these high exposure levels in rural communities. The US Environmental Protection Agency (US EPA) has established a set of standards that are designed to protect human health. The standard for optimal air quality is 0 to 35.4 PM₂.₅ with anything above being rated from unhealthy to hazardous. Seeley Lake, MT experienced unprecedented levels of smoke exposure from July 31 to September 18, 2017. Seeley Lake had 35/50 days with > 200 PM₂.₅ with 70% of days between the range of very unhealthy (150.5 to 250.4 PM₂.₅) and 9 days of > 1000 µg/m³ which exceeds hazardous levels (250.5 to 500.4 PM₂.₅). Though there has been some literature evaluating the health effects of wildfire smoke in the wildland firefighting context, there have not been a lot of studies evaluating the effects on local communities. The present study is designed to assess long-term effects of wildfire smoke through a series of screenings. We used a variety of tests including spirometry, blood pressure, serum markers of inflammation and a variety of other tests to measure changes in human health. The Seeley Lake cohort consisted of 95 participants all of which lived in Seeley Lake and had an average age of 63 years old. Spirometry was one of the main tests used to access changes between the screening in 2017 to the screening in 2018. The FEV1/FVC ratio is commonly used in studies as a good measurement of lung function as it is able to determine the difference between an obstructive or restrictive lung disease. The FEV1/FVC ratio is the amount of air exhaled in the first second divided by all of the air exhaled during a maximal exhalation. Overall, we found that there was a significant decrease in the FEV1/FVC ratio for all participants. A decrease in FEV1/FVC indicates that there is some airway obstruction. We are currently still reviewing the other data collected and assessing potential correlations with the spirometry data. The findings thus far have implications that wildfire smoke can have long lasting effects on human health.

1110 PM₂.₅ Characterization in Biomass and Non-biomass Burning Households
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Exposure to indoor air pollution is a global public health disparity issue. Nearly 3 billion people are exposed to biomass smoke with women and children of low socioeconomic status disproportionately impacted. Fine particulate matter (PM₂.₅) is a component of indoor air pollution with well-established health effects and is significantly elevated in residences burning biomass fuel compared to homes using a non-biomass fuel source. Thus far there is limited exposure-response data establishing reduced health impacts from alternative fuel use compared to biomass burning and the characterization of these complex environmental mixtures beyond concentration is poorly understood. We are preforming a robust characterization of PM₂.₅ from biomass and non-biomass burning households through concentration, composition, oxidative potential, and bioactivity assessments. As part of the Prospective Urban and Rural Epidemiological (PURE-Air) study, PM₂.₅ was collected with a stationary monitor in the kitchen and personal monitors on female and male participants in 24 households in Belupalle and Kheri, India. Filters were stratified between 24 households in Belupalle and Kheri, India. Filters were stratified between 24 households in Belupalle and Kheri, India. Filters were stratified between 24 households in Belupalle and Kheri, India. Filters were stratified between 24 households in Belupalle and Kheri, India. Filters were stratified between 24 households in Belupalle and Kheri, India. Filters were stratified between 24 households in Belupalle and Kheri, India. Filters were stratified between 24 households in Belupalle and Kheri, India.

1111 High-Carbohydrate Oral Load Increases Arrhythmia and Alters Cardiovascular Function One Day after a Single Eucalyptus Smoke Exposure in Sprague-Dawley Rats
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Multiple studies have demonstrated the severe health effects of a single exposure to wildfire smoke. Stemming from underlying physiological changes, these effects include not only acute respiratory complications but also adverse cardiovascular events. Notwithstanding the brevity of exposure and lack of data on the many of these changes, a recent report sheds light on the host susceptible to subsequent stressors. As such, consumption of a high-carbohydrate (HC) meal has been shown to increase stress in the body. Therefore, this study was conducted to determine the cardiovascular effects of a single exposure to eucalyptus smoke and subsequent priming to a HC meal challenge. Adult male Sprague-Dawley rats (14-26 weeks) were exposed to either filtered air (FA) or 0.7 mg/m³ of flaring eucalyptus smoke (ES) for one hour. Animals were fasted starting 15 hours after exposure and then 21 hours later challenged with either vehicle (Veh) or HC emulsion via oral gavage to mimic a high-carbohydrate meal. One hour after gavage, rats were anesthetized and implanted with an intraventricular Millar probe. Rats were then treated with phenylephrine (pressor) followed by sodium nitroprusside (depressor) to determine cardiac and arterial function, and baroreflex sensitivity (BRS). Baseline aortic blood pressure was significantly decreased in ES-Veh rats, whereas left ventricular pressure (LVP) was significantly increased only in ES-HC rats. On the other hand, only the FA-HC group had increased arrhythmogenicity. Lastly, although the BRS pressor response showed a trend towards decreased sensitivity in ES-Veh and FA-HC rats, there were no significant differences. In contrast, both ES-Veh and ES-HC caused a significant blunting of the BRS depressor response when compared to FA-Veh. These results demonstrate that a HC meal increases cardiac pressure and decreases BRS one day after ES exposure. Although these findings of HC after ES smoke exposure indicate heightened cardiovascular risk, it appears that HC and ES on their own also cause cardiovascular dysfunction. In addition, these results suggest that even though ES and HC are likely deleterious on their own, their combined effect is probably most pronounced in hosts with underlying cardiovascular disease. Disclaimer: This abstract does not reflect US EPA policy.

1112 Acute Eucalyptus Smoke Inhalation Sensitizes Rats to the Postprandial Effects of a High Carbohydrate Oral Load
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Previous studies have shown that air pollution exposure primes body systems to heightened responses to everyday activities that stress the cardiovascular (CV) system. The goal of this study was to investigate the impacts of a one-time exposure to eucalyptus smoke (ES) on the postprandial CV effects after a single high carbohydrate (HC) challenge, a cardiometabolic stressor long used to predict CV risk. Three-month-old male Sprague Dawley rats were exposed once (1 hr) to filtered air (FA) or ES (700 µg/m³ fine particulate matter), a key wildland fire air pollution source, generated by burning eucalyptus in a quartz tube furnace. Rats were then fasted overnight, and subsequently administered an oral gavage of either water or a HC suspension (70 kcal% from carbohydrate), mimicking a HC meal, 24 hr post-exposure. Two hours post gavage, cardiac and superior mesenteric artery function, circulating lipids and hormones, and pulmonary and systemic inflammatory markers were assessed. ES inhalation alone caused an increase in glucose, serum interleukin (IL)-13, and keratinocyte chemoattractant (KC)/growth-regulated oncogene (KC-GRO) compared to water in both FA- and ES-exposed animals. By contrast, only ES-exposed and HC-challenged animals had an increase in serum IL-4 and IL-6 compared to FA-exposed animals that received water. Echocardiography data is currently being analyzed. In summary, exposure to eucalyptus smoke can heighten cardiovascular risk, it appears that HC and ES on their own also cause cardiovascular dysfunction. In addition, these results suggest that even though ES and HC are likely deleterious on their own, their combined effect is probably most pronounced in hosts with underlying cardiovascular disease. Disclaimer: This abstract does not reflect US EPA policy.
Inhalation of Ozone (O₃), a main component of smog, adversely affects lung function. We have shown that O₃ exposure leads to activation of pulmonary macrophages toward an M1/pro-inflammatory phenotype, which is dependent on NF-κB function. Nitric oxide is a known regulator of NF-κB function, either via direct nitrosylation of the p65 subunit or indirectly via regulation of translocation. However, there were a number of other targets for NO within the lung that exacerbate inflammation. Here we investigated the effects of 3 different S-nitrosothiole (SNO) donors (ethyl nitrite, ENO, S-nitroso-N-acetyl cysteine, SNAC, and S-nitrosopropanamide, SNOPPM) on inflammatory cell recruitment and activation. In addition, we have developed a human exposen model to examine macrophage activation. Female mice were exposed to 0.8 ppm of O₃ or air for 3 hours and given 0.0125% ENO (50µg), or 50 ng of SNAC or SNOPPM intranasally 1hr following exposure. Mice were sacrificed 48hr following dosing. Alveolar and tissue associated macrophages were obtained via bronchoalveolar lavage and lung digestion. Lung lobes were either inflamed for histology or snap frozen. Immunohistochemical staining of paraffin-embedded tissue sections allowed for assessment of lung injury and inflammatory signaling. O₃ increased BAL protein, which was opposed by ENO and SNOPPM but not SNAC. O₃ did not significantly alter the cell content of either the tissue or the BAL, but all SNO donors increased total recruitment and particularly within immature unactivated macrophages. ENO reduced the expression of markers of epithelial cell damage, lipid peroxidation, and downstream inflammatory signals. All SNO donors reduced O₃ induced expression of the pro-inflammatory marker Ly6C in alveolar and tissue associated macrophages. All donors reduced the O₃ induced appearance of YM1 in tissue associated macrophages. These studies imply that SNO donor administration inhibits macrophage activation with SNOPPM being the most effective. This conclusion is supported by in vitro work that shows SNOPPM can oppose LPS-mediated induction of NF-κB activity. In our human studies, we have examined the effects of low dose O₃ exposure while exercising for 3 hours on pulmonary inflammatory activation. Examination of induced sputum reveals an inflammatory activation pattern that we have not seen in our mouse model. Our data show O₃ increases pro-inflammatory signaling in humans and animals and that inhaled SNO donors may be able to oppose this process, leading to decreased epithelial injury.

Wood smoke exposure causes airway inflammation and oxidative stress, which contributes to cardiopulmonary disease and early mortality; however, the molecular mechanisms responsible for these adverse effects are unclear. To date, epithelial cell monocultures have been widely used for identifying the molecular mechanisms involved in toxicity. While epithelial cells serve as the barrier between the host and the environment, they are only one of many cell types within the airway that cooperatively affect lung function. We hypothesized that exposure to wood smoke condensate (WSc) would induce both the oxidative stress and pro-inflammatory response in airway fibroblasts, which reside immediately beneath the airway epithelium and play a critical role in maintaining tissue homeostasis. To test this hypothesis, we developed a low-serum Transwell-based in vitro organotypic model that recapitulates in vivo exposures in which airway epithelial cells (16HBE) are directly exposed and airway fibroblasts (IMR90) are indirectly exposed. We determined that exposure to WSc at 20 µg/cm² did not decrease trans-epithelial electrical resistance (TEER) or increase permeability of the epithelial layer to fluorescein-labeled dextran (20kDa) following 24 hours of exposure, indicating that our exposure conditions do not disrupt epithelial barrier functions. We then assessed the effects of WSc dose (10 and 20 µg/cm²) and the kinetics of the exposure response (2, 4, 6, 8, 10, 12, and 24 hours) on the expression of the sentinel pro-inflammatory and oxidative stress genes interleukin-8 (IL-8) and heme oxygenase (HMOX1), respectively. Direct exposure of epithelial cells induced peak IL-8 (3-fold) and HMOX-1 (15-fold) gene expression at 6 and 8 hours of exposure, while the indirectly exposed fibroblasts exhibit peak IL-8 (2.5-fold) and HMOX-1 (25-fold) gene expression at 8 and 4 hours of exposure, respectively. These results demonstrate distinct gene expression kinetics of epithelial cells and fibroblasts in response to WSc exposure. The discrepancy in gene expression kinetics may suggest that epithelial cell signaling influences the stress response of underlying fibroblasts. The influence of signaling between cells in this organotypic model facilitates new insights into the origins of cardiopulmonary diseases in response to wood smoke exposures.

Human exposure to ozone at peak ambient levels is associated with an accumulation of activated macrophages in lung and airways. How these cells influence ozone-induced pulmonary morbidity and neutrophilic inflammation remains unclear. Following exposure of rodents to ozone, a persistent increase in proinflammatory macrophages is observed in the lung. Moreover inhibiting the activity of these cells mitigates toxicity. We hypothesize that proinflammation macrophages play a similar role in human toxicity. To address this, we assessed the phenotype of macrophages in induced sputum following ozone exposure. Healthy men and women were exposed for 3 hr to clean air and to 0.2 ppm ozone two weeks apart in a randomized cross-over design. During exposures mild to moderate exercise was performed. Subjects were initially screened for their ability to produce sputum, which was used as a baseline for comparison with induced sputum collected 24, 48 or 72 hr after exposure. Mucus plugs were manually separated from sputum, weighed, and incubated in 0.1% dithiothreitol. After counting, recovered cells were processed for histological analysis and flow cytometry. A gating strategy using successive bivariate scatter plots of forward/side scatter, CD45⁻/⁺, live/dead analysis, and relative CD14/CD16 positivity identified 3 discrete populations of cells: macrophages, monocytes and neutrophils. Cell uniformity in each region was verified by cell sorting and microscopy. In contrast to paired baseline and room air control, a significant increase in mature proinflammatory (M1) CCR2+ Macrophages was observed in samples collected from subjects 24 and 48 hr after ozone exposure in 5 of 6 subjects, a response which was reduced at 72 hr. This was correlated with increases in neutrophils and immature macrophages. These findings demonstrate that CD11b/CCR2 expression on macrophages after non-invasive sputum collection can iden-
1117 Differential Gene Expression in Alveolar versus Intratissial Macrophages during Ozone-Induced Pulmonary Inflammation

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Ozone (O3) is a criteria air pollutant that causes pulmonary inflammation and injury. Lung resident macrophages termed ‘alveolar macrophages’ (AMs) contribute to the O3-induced pulmonary inflammatory response. However, pulmonary macrophages are a heterogeneous population and it is currently unknown how the other macrophage populations contribute to and/or mitigate O3-induced inflammation. Intratissial macrophages (IMs) are a population of pulmonary macrophages present in the lung parenchyma. However, the role of IMs in O3-induced pulmonary inflammation and injury is unknown. We hypothesized that IMs attenuate O3-induced pulmonary inflammation by upregulating pathways important for resolving lung injury. Female C57/Bl/6j mice were exposed to filtered air (FA) or O3. Whole lung tissue was excised 24 hr and 48hr after exposure to isolate macrophage populations. Using fluorescence activated cell sorting (FACS), we performed quantitative PCR (qPCR) in whole lung macrophages (CD11b+Ly6G+Ly6C+CD11b+SiglecF+) and IMs (CD45−CD124+Ly6GCD64+CD41b+). Macrophage RNA was isolated to analyze expression of receptors known to be important in O3-induced pulmonary inflammation (Scarb1, Cd36, Cd163) and expression of markers known to participate in the resolution of lung injury (Merk, Tgfb, Cd36). CD163 expression was unchanged after O3; however, IMs had increased baseline Il10 expression when compared to AMs that was significantly upregulated after O3. Scarb1 and Cd163 expression in AMs was unchanged after O3 whereas IMs had significantly higher expression of Scarb1 and Cd163 compared to AMs that was augmented after O3. Neither AMs nor IMs had alterations in expression of Merk, Tgf-β, and Cd36 after O3 compared to FA controls. These data indicate that IMs are involved in the pulmonary inflammatory response to O3 through upregulated expression of Il-10 and scavenger receptors. Future RNA sequencing studies will determine the molecular pathways responsible for these alterations in gene expression in IMs.

1118 Pathologic Effects of Depletion of CD11b+ Monocytic Cells in Mouse Lung following Ozone Inhalation

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Ozone (O3) is a ubiquitous urban air pollutant known to cause lung inflammation, alveolar epithelial damage and oxidative stress. CD11b+ macrophages have been shown to accumulate in the lung following ozone exposure; these cells have been characterized as Ly6C+ proinflammatory and Ly6C− anti-inflammatory. To assess the role of CD11b+ macrophages in ozone toxicity, we used mice with a transgenic diphertheria toxin (DT) receptor under the control of the CD11b promoter (CD11b-DTR). Mice were treated i.p. with 25 μg/kg DT to deplete CD11b+ monocytes. After 18 h, mice were exposed to air or 0.8 ppm O3 for 3 h. IL-10, TNFα, IL-6, and LPS were elevated when compared to LPS treated mice; conversely, the percentage of resident alveolar macrophages (CD45+CD11b+SiglecF+CD44+) was reduced. These results suggest that pre-exposure to ozone primes the lung for sepsis-induced ALI in mice. The ALI appears to be mediated, in part, by loss of resident alveolar macrophages and infiltrating neutrophils and monocytes are pro-inflammatory macrophages into the lung. Supported by NIH ES004738, ES005022, SOT Donald E. Gardner Inhalational Toxicology Education Award.

1119 Ozone Exposure Predisposes Mice to Sepsis-Induced Lung Injury

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Ozone is a ubiquitous urban air pollutant known to cause alveolar epithelial barrier dysfunction. Recently, US Environmental Protection Agency (US EPA) compliant levels of ozone exposure have been directly linked to increased incidence of human acute respiratory distress syndrome (ARDS), a severe form of acute lung injury (ALI). While sepsis is a well-established cause of ARDS, it is unknown why only certain individuals develop ARDS as a consequence of sepsis. Our goal was to analyze the potential role of ozone pre-exposure in the development of sepsis-induced ARDS. Male C57/Bl/6j mice were exposed to 0.8 ppm ozone or air in a whole-body exposure chamber for 3 hr followed 24 hr later by i.v. administration of lipopolysaccharide (LPS) (2.4-3 mg/kg) to model bacterial sepsis, or phosphate buffered saline (PBS) control. Mice were euthanized 24 hr later and bronchoalveolar lavage fluid (BAL) fluid and cells collected and analyzed for markers of lung damage (protein, IgM) and leukocyte phenotype. BAL protein content and IgM levels from mice exposed to ozone and LPS were elevated when compared to LPS or ozone alone. Flow cytometric analysis of BAL leukocytes revealed a significant increase in activated neutrophils (CD45+CD11b+Ly6G+Fla480-Ly6Ch1) in the ozone/LPS group relative to the other groups. A greater percentage of mature pro-inflammatory macrophages (CD45+CD11b+Ly6G-Fla480+CD11c+Ly6Ch1) was also observed in the ozone/LPS treated mice, conversely, the percentage of resident alveolar macrophages (CD45+CD11b+SiglecF+Fla480+) was reduced. These results suggest that pre-exposure to ozone primes the lung for sepsis-induced ALI in mice. The ALI appears to be mediated, in part, by loss of resident alveolar macrophages and neutrophils and monocytes are pro-inflammatory macrophages into the lung. Supported by NIH ES004738, ES005022, SOT Donald E. Gardner Inhalational Toxicology Education Award.

1120 Estrogen Regulation of Ozone-Induced Lung Inflammation

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Aspiration is a lung disease characterized by exaggerated lung inflammation that is exacerbated upon air pollution exposure. The risk of an asthma exacerbation requiring a hospital admission is almost twice as high for women than men. Environmental and physiological factors, including exposure to ozone and circulating levels of sex hormones, have been shown to increase the risk of asthma exacerbations. However, the mechanisms involved remain unknown. Our lab has recently challenged male and female C57/Bl/6j mice with 2 ppm of ozone for 3 h and identified sex differences in lung inflammation and airway hyperresponsiveness. To test the hypothesis that circulating estrogen levels can regulate lung function in response to ozone, we performed gonadectomy and hormone replacement (17β-estradiol, 2 weeks) in adult male and female mice. In control females, the stages of the estrous cycle were monitored by daily by vaginal smear, and confirmed by serum sex hormone levels. We exposed animals to 2 ppm ozone or filtered air (FA) for 3 hours, and we compared lung function parameters at 24 h after exposure by methacholine challenge using the FlexiVent system. We found significant changes in respiratory parameters in males and females, and in females exposed to ozone at different stages of the estrous cycle. Gonadectomized males exposed to ozone had increased resistance (Rs) and elastance (Es) than females and males exposed to FA, and treatment with estradiol decreased lung expansion in both sexes. Female mice exposed to FA in the metestrus and diestrus stages exposed had higher Rs and Es values than when compared to the proestrus and estrus stages. Contrarily, exposure to ozone caused a decrease in these parameters during metestrus and diestrus, but a slight increase in the proestrus and estrus. These results suggest that pulmonary function following ozone exposure is affected by circulating hormone levels. This information could lead to better individualized treatment options for men and women suffering from asthma, and identification of sex-specific environmental and hormonal triggers that lead to severe disease symptoms.
1121 Neuroendocrine Stress Axis and Adaptation after Repeated Daily Ozone Exposure


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After repeated daily ozone exposure for more than 2 days, rodents demonstrated total/circulation/circulation from internal lung injury, inflammation, and functional alterations. The mechanisms of adaptation to ozone, other pollutants, or non-chemical stressors are not well known. We hypothesized that since ozone-induced lung injury and inflammation are mediated through the activation of neuroendocrine pathways, the adaptive response to initial lung injury and inflammation might involve the lack of neuroendocrine response, and that low level 1-month exogenous glucocorticoid treatment might result in steroid resistance and impairment of adaptation. Male Wistar Kyoto rats (12-week old) were treated with saline or dexamethasone sul fate (DEX, 0.01 mg/kg/day; 7 days/week for 4 weeks); ip prior to and during daily exposure to air or 0.8 ppm ozone for 2 or 4 days followed immediately by injury/inflammation and systemic neuroendocrine changes were assessed post-exposure. Body, thymus and spleen weights were lower in animals treated with DEX when compared to saline controls. DEX reduced circulating adrenocorticotropic hormone in all animals, however, it had no effect on prolactin (PRL), luteinizing hormone (LH) or thyroid stimulating hormone (TSH) levels. Ozone exposure for 2, but not 4 days, was associated with a remarkable depletion of PRL, LH and TSH regardless of DEX treatment. DEX-treatment was associated with ~80% depletion of circulating corticosterone in all air-exposed rats. Ozone exposure for 2, but not 4 days, reversed this depletion, suggesting that adaptation was associated with reduction of glucocorticoid hyroid. Ozone-induced hypoxic, glucose intolerance and inhibition of insulin release in response to glucose noted after 2 days were not present after 4 days, indicating adaptation of metabolic response. This adaptation was not influenced by DEX. The lung injury/inflammation and increased lavege fluid IL-6 levels noted after the 2nd day of ozone exposure was not observed after the 4th day of exposure in saline-pre-treated rats, however, this adaptation was less pronounced in DEX-treated rats. These results demonstrate that adaptation to repeated ozone exposure is not in only pulmonary but also neuroendocrine pathways, and that long-term steroid pretreatment had a small effect on the adaptation to lung injury and inflammation. Disclaimer: Does not reflect US EPA policy.

1122 Dynamic Changes in Neuroendocrine Hormones and Inflammatory Cell Egress following an Acute Ozone Exposure in Rats

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Acute exposure to ozone leads to multi-organ alterations through activation of the sympathetic-adrenal-medullary and hypothalamic-pituitary-adrenal axis, which control the synthesis and release from adrenal glands of epinephrine and glucocorticoids, respectively. These hormones regulate and maintain homeostasis through modulating changes in two fundamental survival processes in the body: metabolism and immune response. The purpose of this study was to elucidate the temporal sequence behind ozone-induced metabolic alterations and innate immune responses in the lung by constructing a time line of events for stress hormone release and egress of immune cells. Male, 12-14 weeks old Wistar-Kyoto rats were exposed to filtered air or ozone (0.4 or 0.8 ppm) for 30 min, 1h, 2h, or 4h followed immediately by necropsy to collect blood and tissues for analysis. Circulating hormones were measured in plasma or serum samples. The levels of thyroid stimulating hormone, luteinizing hormone, and prolactin steadily decreased with increased exposure times to 0.8 ppm ozone. Adrenocorticotropic hormone, which stimulates the release of corticosterone from the adrenal cortex, was increased at 30 min (p=0.0759), 1h, and 2h. This was prior to the elevation of corticosterone at 1h, 2h, and 4h-post-exposure to 0.8 ppm ozone. Ozone exposure (0.8 ppm) led to significant increases in epinephrine levels at the 1h, 2h, and 4h timepoints, illustrating the rapid response of these stress response pathways. These findings corresponded with significant increases in free fatty acids (1h, 2h, and 4h), total cholesterol (1h; and blood glucose levels (4h) following exposure to the high dose of ozone. Furthermore, ozone exposure at 0.8 ppm led to significant decreases in circulating white blood cells and lymphocytes at the 4h timepoint. Neither apoptotic nor necrotic white blood cell subpopulation levels were altered, suggesting mobilization into tissues rather than cell death was responsible for the decreased count. Collectively, these findings illustrate the temporal pattern of effects that lead to the release of neuroendocrine hormones, metabolic alterations, and immune responses following ozone exposure. These dynamic datasets are critical for the accurate characterization of injury mechanisms as well as the development of adverse outcome pathways associated with exposure to environmental stressors. Disclaimer: Does not reflect US EPA policy.

1123 Ozone Increases Plasma Kynurenine and Impacts Hippocampal Serotonin Receptor and Neurotrophic Factor Expression: Role of Stress Hormones

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Air pollution is associated with increased incidence of neurological and mental health disorders, but underlying mechanisms remain unclear. Recent work has shown that air pollutants activate the hypothalamic-pituitary-adrenal (HPA) axis, releasing stress hormones (glucocorticoids) that exert profound effects on many biological systems. Stress can affect cognition, mood, and behavior, and chronic stress can produce biochemical and structural changes that contribute to disease processes in the brain. However, the role of stress hormones in mediating impacts of air pollutants on the brain is unknown. The objective of the present study was to assess effects of ozone on biological factors relevant to depression and dementia, notably serotonin signaling and neurotrophic factor expression, and examine the role of stress hormones in mediating these effects. Male Fischer-344 rats (n=5/group) were exposed by nose-only inhalation to air or 0.8 ppm ozone for 4 h with or without metyrapone, a drug that blocks production of the glucocorticoid corticosterone. Plasma levels of the serotonin precursor tryptophan and the tryptophan metabolite kynurenine, and changes in gene expression in the hippocampus, a stress-sensitive region of the brain implicated in memory, cognition, and depression, were assessed. Ozone increased the ratio of kynurenine to tryptophan (2.7-fold, p<0.001), a feature of a number of brain, inflammatory, and metabolic disorders. Serotonin receptor genes were differentially affected by ozone, while mRNA levels of neurotrophic factors were decreased. Some, but not all, of these effects were blocked by metyrapone (Ozone x Metyrapone factor interactions, p<0.05) and reproduced by administration of corticosterone (r=0.83, p=0.01), establishing a role for stress hormones in mediating effects of ozone on the brain. The findings show that short-term exposure to a commonly air pollutant implicated biological processes implicated in depression and cognitive disorders, and contribute to the weight of evidence linking exposure to air pollutants and effects on the brain.

1124 The Impact of Ozone Exposure and Sedentary Lifestyle on Microglia and Mitochondrial Bioenergetics of Female Long-Evans Rats

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Ozone is a widespread and highly reactive air pollutant that produces cardiovascular and pulmonary dysfunction. We sought to assess neurological changes in glial morphology and mitochondrial bioenergetics in response to both sedentary lifestyle and ozone exposure. Astrocytes work intimately with neurons to sustain functioning synapses as well as preserve the blood brain barrier. Microglia are highly plastic and aid in safeguarding the brain by removing damaged neurons, plagues, and xenobiotics. Microglia are key regulators of cellular energy homeostasis and may play a role in mechanisms that regulate the temporal sequence behind neurodegeneration. To develop an animal model in which female Long-Evans rats were either sedentary or active (exercise on running wheels) starting at postnatal day (PND) 22 until the age of PND 100 and then exposed to O3 (0.0, 0.25, 0.5 or 1.0 ppm) 5 h/day for two consecutive days. Immediately following O3 exposure, the rats were sacrificed and brains were either fixed and stored at 4°C or dissected on ice, quick frozen, and stored at -80°C until analysis. For astrocytes (GFAP) and microglia (Iba1) immunohistochemistry, we measured the area of coverage and reactive morphology, respectively, in the hypothalamus (HYP) and hippocampus (HIP), two brain regions with known roles in coordinating stress responses. We found more reactive microglia within the hippocampus (HYP) and hippocampus (HIP), two brain regions with known roles in coordinating stress responses. We found more reactive microglia within the hippocampus (HYP) and hippocampus (HIP), two brain regions with known roles in coordinating stress responses. We found more reactive microglia within the hippocampus (HYP) and hippocampus (HIP), two brain regions with known roles in coordinating stress responses. We found more reactive microglia within the hippocampus (HYP) and hippocampus (HIP), two brain regions with known roles in coordinating stress responses. We found more reactive microglia within the hippocampus (HYP) and hippocampus (HIP), two brain regions with known roles in coordinating stress responses. We found more reactive microglia within the hippocampus (HYP) and hippocampus (HIP), two brain regions with known roles in coordinating stress responses.
Acute ozone (O3) inhalation in rats induces firing of catecholaminergic neurons in the nucleus tractus solitarius (NTS) in the brainstem as well as paraventricular nucleus (PNV) of hypothalamus. O3 exposure increases circulating stress hormones through activation of sympathetic-adrenal-medullary and hypothalamic-pituitary-adrenal axes. Adrenalectomized (AD) rats lacking circulating stress hormones epinephrine (EPI) and corticosterone (CORT) have attenuated pro-inflammatory responses to O3. We hypothesized that O3-induced changes in the circulating levels of pituitary-derived and other neuroendocrine hormones will correlate with changes in global gene expression in the brainstem and hypothalamus. Additionally, based on our previous pulmonary findings, we investigated the effects of AD on modifying O3-induced changes in pituitary hormones as well as gene expression. Male Sprague-Dawley rats (12 wks) that underwent sham surgery (SH) or AD were exposed to O3 (0.8 ppm) or filtered air, 4 hr/day for 1 day. Serum pituitary hormones were quantified, and mRNA from brainstem and hypothalamus was isolated, purified and sequenced. O3 exposure decreased the serum levels of thyroid-stimulating hormone (TSH), prolactin (PRL), and luteinizing hormone (LH) in SH rats. O3-induced TSH and PRL but not LH reductions were prevented in AD rats. AD increased ACTH nearly 5-fold in all animals. O3-exposure in SH rats significantly changed gene expression in the brainstem and hypothalamus (303 and 568 genes, respectively). Virtually no genes were changed after O3 exposure in AD rats. AD in air-exposed rats changed only ~18 genes in the brainstem and hypothalamus. O3 induced enrichment of genes involved in activation of hedgehog signaling, response to alpha interferon, response to low oxygen levels, and activation of mTORC1, among others. Gene expression altered in both tissues was analogous to those altered by glucocorticoids and L-dopa, suggesting a pivotal role of CORT and neurotrophins. These findings suggest that O3 inhalation promotes a rapid rearrangement of gene expression in the brainstem and hypothalamus, which reflects changes in biological processes such as hypoxia and/or inflammation; and results in activation of neuroendocrine axes. Elimination of circulating stress hormones abolishes O3-induced changes in brain gene expression and neuroendocrine activation. Disclaimer: This abstract does not reflect US EPA policy.

Ozone (O3) is one of The US EPA’s six “criteria air pollutants” due to its association with adverse effects on human health including the exacerbation of existing respiratory diseases and heightened susceptibility to respiratory infections. Inhalation to O3 rapidly reacts with airway lining fluid constituents to form biologically active molecules that trigger inflammation and impair host defense by altering innate immune responses. Beyond the initial O3-airway surface reactions, the mechanisms by which O3 elicits inflammation and impairs host defense are not known. Extracellular vesicles (EVs) are nano-scale lipid membrane bound particles known to function as intercellular messengers and carry cargo such as microRNAs (miRNAs) that can regulate mRNA expression in recipient cells. We hypothesized that O3 alters miRNAs carried by EVs between airway cells (e.g., epithelia and macrophages), thereby altering gene expression in the airways in a manner that contributes to inflammation and innate immune dysfunction. In this study, we exposed female C57BL/6 mice to O3 (~10x ambient) or filtered air (FA) for 3 h in whole body chambers. Bronchoalveolar lavage fluid (BALF) was collected, EVs isolated and their content assessed. We found that MVs and Exos were released into BAL following O3 exposure. Flow cytometric analysis showed that MVs were mainly derived from type I epithelial cells (ATI), while Exos were from type II epithelial cells (ATII). Furthermore, the majority of the cargo carried by ATI cell-derived MVs (ATI-MVs) consisted of smaller RNAs. In contrast, there were negligible amounts of small RNAs in ATII cell-derived Exos. In ATI-MVs, small RNAs, but not proteins or large RNAs, were robustly increased after O3 exposure. Using microRNA (miRNA) arrays, epithelial MVs generated following ozone exposure were found to encapsulate a selected miRNA repertoire. These findings suggest a pathway for selective loading of miRNAs into MVs in response to O3. We also found that MV-miRNA-derived miR-185 induces ATI cell necroptosis via augmenting caspase 8-mediated signaling pathways. Collectively, these findings demonstrate that O3 induces the generation of miRNA containing by lung epithelial cells; moreover these play a role in epithelial cell death. Supported by NIH ESO04738 and ESO050202; R01 GM127596, R33 AI121644 and R01 GM111313.

Airway Transcripomic Responses to Ozone: Altered Extracellular Vesicle MicroRNA and Alveolar Macrophage mRNA Expression Profiles

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Air pollution (AP) is a leading global risk factor for cardiovascular and metabolic-related morbidity and mortality. Particulate matter that is <2.5 micrometers (PM2.5) is a persistent pollutant and is monitored by environmental agencies around the world using satellite and ground-level methods. Populations in areas with high levels of ambient air pollution likely have increased risk of developing metabolic syndrome. Understanding the molecular mechanisms underlying this multifactorial disease is critical for developing population level preventive approaches. We have previously demonstrated that chronic exposure to PM2.5 causes insulin resistance in mice. Here, we modeled early childhood PM2.5 exposure using 3-week-old C57BL/6J mice (male and female). Mice were exposed through inhalation to concentrated PM2.5 (~10x ambient) 3 h/day, 5 days/week, over 14 weeks. The control mice received filtered air (FA) from the same concentration system. All mice were randomized to a normal or high-fat diet in order to model air pollution with the background of an increased body weight and high fat intake. We found that mice that experienced 14-weeks of PM2.5 exposure had impaired glucose metabolism, compared to FA, as measured by glucose and insulin tolerance testing. In contrast, female mice exposed to PM2.5 did not exhibit similar metabolic perturbations. Using a comprehensive and systematic transcriptome map, we identified differentially expressed genes in the liver, visceral white adipose, brown adipose, and skeletal muscle. By incorporating open chromatin signatures, we have discovered widespread decrease of chromatin accessibility in liver upon the PM2.5 exposure. We also performed transcriptome mapping on brain tissue, particularly on the hypothalamus and rostral ventrolateral medulla (RVL/M) tissue, which is both associated with the cardiovascular function. The male mice exhibited phenotype reversal 8 weeks after cessation of PM2.5 exposure, and we conducted transcriptome and chromatin accessibility analysis to find the potential mechanism of transcription reprogramming. DEGs discovered in PM liver and WAT have been majorly
1129 Behavioral Alterations with Ambient Ultrafine Particulate Matter Exposure in Aged Alzheimer’s Disease Mice


Alzheimer’s disease (AD) is a neurodegenerative disorder that diminishes memory and cognitive skills as well as movement and olfactory function. The major pathological components of AD are sparsely distributed amyloid plaques and neurofibrillary tangles. Environmental factors like air pollution may contribute to the overall risk of disease development. Of concern is exposure to ultrafine particles (UFP, <0.1 μm in aerodynamic diameter), as they deposit efficiently in all regions of the respiratory tract, evade clearance, and can translocate to secondary organs, where they may induce inflammation. We hypothesized that triple transgenic AD mice (3xTgAD) exposed to concentrated ambient UFPs undergo accelerated AD progression and altered performance in tests of cognitive function, as well as non-memory-related behavioral tasks. UFPs from ambient air were concentrated using the Harvard Ultrafine Concentrated Ambient Particle System (HUCAPS). Cohorts of male mice were exposed at 12.5–13 months of age, when AD pathology is present, for 2 weeks (4 hours/day, 4 days/week) to filtered air or HUCAPS (number concentration 1.22E+5/cm³, count median diameter 79 nm, geometric standard deviation 1.5). Following exposure, behavioral testing was performed to assess spatial learning and memory, recognition memory, locomotor and olfactory function, and motivation. Radial arm maze testing showed that 3xTgAD mice had diminished spatial learning and working memory as compared to non-transgenic (NTg) mice; no dependence on exposure was found. No effects were observed with reference memory, recognition memory, or locomotor activity. 3xTgAD mice also showed diminished olfactory discrimination compared to NTg mice. Progressive ratio testing of motivation showed a significant interaction between genotype and treatment such that motivation was diminished in 3xTgAD mice with HUCAPS exposure but enhanced in NTg mice. While there was a significant effect of HUCAPS-exposure on motivation, our overall results show that the predominating effect observed at this age - when pathology is present - is AD-related and detectable in multiple behavioral domains (spatial learning, working memory, olfactory discrimination). The inability to detect HUCAPS related effects in other behavioral paradigms indicates that HUCAPS doesn’t alter the cognitive trajectory of AD progression in this mouse model. NIH R01ES020332, T32ES007026, P01ES001247.

1130 The Effects of Ultrafine Particulates from Air Pollution on the Progression of Amyloid Pathology in Alzheimer’s Disease


Currently, 44 million people are affected by Alzheimer’s Disease (AD) worldwide and the number continues to rise. While a small percentage of cases can be accounted for by specific gene mutations, the majority are thought to arise from gene-environment interactions. Exposure to air pollution has been identified as a possible environmental factor contributing to the disease. We hypothesize that exposure to ultrafine particulates (UFP, <100nm) from air pollution enhances the progression of amyloid pathology and the formation of amyloid-β plaques, an important indicator of disease. Two cohorts of 3xTgAD mice, a model for AD, and non-transgenic mice were exposed to concentrated ambient UFP (Harvard Ultrafine Concentrated Ambient Particle System, HUCAPS). Each cohort was exposed for 4 hours a day, 4 days per week for 2 weeks at 12 months of age. The mean particle concentration ranged from 3.39x10⁵/cm³ to 9.01x10⁵/cm³ and the count median diameter from 81±12 nm to 84±4 nm (Geometric Standard Deviation 1.45-1.53). One cohort of mice was sacrificed immediately after exposure, while the other recovered for 2 months. One half of each cohort was used to harvest brain tissue via microdissection, while the other half was perfused and dedicated to immunofluorescence analysis. The sectioned brains were stained with 6E10, which detects mature amyloid-β and precursors like amyloid precursor protein (APP). There was no indication of lung inflammation based on bronchoalveolar lavage fluid cellular and biochemical analyses. No significant changes in 6E10 hippocampal staining were found between 3xTgAD mice exposed to concentrated UFP and the control 3xTgAD mice exposed to filtered air in either cohort. When insoluble amyloid-β42 protein was measured in the microdissected hippocampi, the 3xTgAD HUCAPS mice trended towards increased levels (p=0.053) in comparison to the 3xTgAD filtered air mice within the immediate cohort. This trend was not sustained in mice that were allowed to recover for 2 months. These findings suggest that short-term, near-ambient exposures to concentrated UFPs do not directly alter amyloid pathology once plaques have already formed. Funding: NIH R01ES020332, T32 ES007026, ES001247.

1131 Traffic-Generated Air Pollution Exposure Mediates Adipocyte Hypertrophy Associated with Increased Angiotensin II Signaling in C57Bl/6 Wildtype Mice

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Recent studies have reported a positive correlation between exposure to environmental air pollution and metabolic syndrome and/or obesity in both children and adults. Additionally, at least a few of these studies have reported traffic-generated pollutants are associated with increased incidence of obesity, associated with cardiovascular disease (CVD). To date, very limited information exists on the effects of inhaled pollutants on adipocytes. The renin-angiotensin system (RAS), when dysregulated, is known to mediate pathogenesis in the cardiovascular system, and in adipocytes, through Angiotensin (Ang) II signaling via the Ang II Type 1 (AT1) or Type 2 (AT2) receptor(s). We have previously reported that inhalation exposure to mixed vehicle emissions (MVE) results in increased plasma Ang II and adipocyte AT-1 expression in C57Bl/6 wildtype mice. Thus, we hypothesize that MVE-exposure results in altered RAS signaling in adipocytes, associated with adipocyte hypertrophy and/or altered adipocyte signaling. To test this hypothesis, 3 mo old male C57Bl/6 mice on either a high-fat “Western” diet (HF, 21% fat) or standard (LF, low fat) mouse chow were randomly assigned to inhalation exposure of either filtered air (FA) or a mixture of 70 µg PM/m³ diesel exhaust + 30µg PM/m³ gasoline exhaust (MVE n=10 per diet) for 6 hr/day for 30 days. MVE exposure resulted in adipocyte hypertrophy, which was associated with increased expression of angiotensinogen (AGT), and the AT1 receptor in adipocytes in MVE-exposed mice compared to FA controls, as determined by immunofluorescence (IF) and immunoblotting. These outcomes were further exacerbated in MVE+HF diet animals, compared to MVE+LF or FA controls. Furthermore, MVE-exposure resulted in increased adipocyte expression of the inflammatory marker, TNF-α, and monocyte/macrophage infiltration (MOMA-2), and a decrease in expression of glucose transporter (GLUT)-4, compared to FA controls. These findings indicate that inhalation exposure to traffic-generated pollutants can promote induction of adipocyte Ang II signaling, macrophage recruitment, and inflammation that are associated with decreased GLUT-4 receptor expression (and possibly impaired glucose uptake), which are further exacerbated by concurrent consumption of a high fat diet. Funded by NIH R01ES020795 to AKL.

1132 DEP-Induced Airway Epithelial Barrier Dysfunction Involves a Reduction in the Tight Junction Protein Tricellulin


Perturbations in the epithelial barrier have been increasingly linked to the pathogenesis of asthma. While airborne particulate matter (PM) has been linked to asthma development and exacerbation, its impact on the function of the epithelial barrier has not been fully assessed. Of particular interest is Tricellulin, a tight junction protein in the same family of Occludin, which localizes specifically to points of tricellular contact and is hypothesized to regulate the permeability of macromolecules across the epithelium at these points. In order to test the impact of diesel exhaust particles (DEP), a major component of PM, on epithelial barrier function and Tricellulin expression, Standard Reference Material (SRM®) 2975 Diesel Particle Matter (DEP) (National Institute for Standards and Technology, Gaithersburg, MD) was suspended in culture media and exposed to nonmonolayers of the human bronchial epithelial cell line 16HBE140. Six-hour treatment with DEP significantly reduced 16HBE barrier function as determined by increased permeability of four ka Da FITC-Dextran (28.2±4.0 versus 19.6±4.34 µg, n=4, p<0.05) and decreased transepithelial electrical resistance (523±6 versus 602±5 Ohms, n=4, p<0.05). This corresponded with a decrease in Tricellulin as measured by Western blot (61±17% normalized band intensity versus vehicle, n=3, p<0.05) without reducing Occludin. To test the impact of DEP on developing lungs, neonatal Balb/c mice (postnatal day 5-7) were exposed to 25±50 µg/m³ aerosolized DEP or filtered air by whole body inhalation for two hours per day for five consecutive days, and sacrificed 2 weeks later. Following these whole-body inhalation exposures, Tricellulin expression was significantly reduced in the lungs as measured by Western blot (65±20% normalized band intensity ver-
sus filtered air, n=7, p<0.05). Taken together, in vitro exposure to DEP caused a significant reduction in epithelial barrier function with a corresponding reduction in Tricellulin expression. A similar reduction was seen in whole lungs of neonatal mice two weeks following exposure to aerosolized DEP. Overall, these results suggest that early life exposure to DEP may have lasting impacts on epithelial barrier structure and function.

1135 Marco Regulates In Vivo Response to Low Molecular Weight Hyaluronan Fragments

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Ozone is criterion air pollutant, which enhances cardiorespiratory morbidity and mortality and exacerbates pre-existing respiratory diseases. Prior work demonstrates that macrophage scavenger receptor (Marco) is critical for the clearance of O3-derived oxidized lipid species. We hypothesize that Marco also regulates biological response to O3-derived low molecular weight hyaluronan (sHA) fragments. We challenged 8-10 weeks old CS7BL6 (WT) and Marco-/- mice with 2ppm O3 or filter air for 3 hours. 24h after exposure mice underwent BAL for total cell count, differentials and hyaluronan measurement. We observed significantly increased level of BAL hyaluronan in O3-exposed Marco-/- mice. Moreover, at 6h following oropharyngeal instillation of sHA (1.5mg/ml) versus saline in Marco-/- or CS7BL6 mice, we observed that Marco-/- mice had increased cell count and neutrophil influx with increased BAL cytokines (IL6, TNF alpha, MCP1 and KC) in comparison to WT. Alveolar macrophages harvested from sHA instilled WT mice significantly increased their Marco expression. Moreover, in time course experiments with alveolar macrophages harvested from naive WT and Marco-/- mice using Rhodamine labeled sHA, we observed enhanced sHA binding in WT vs Marco-/- after 2h of treatment by confocal microscopy. In these same experiments, we observed a positive correlation between level of binding sHA and Marco receptor expression at the cell surface. Finally, we evaluated baseline levels of TR4 and CD14 expression in Marco-/- and WT mice by RTPCR and observed increased RNA for TR4 and CD14 in the Marco-/- alveolar macrophages. In summary, our data suggest a central role for Marco receptor in biological response to low molecular weight hyaluronan. Deletion of this scavenger receptor lead to accumulation of hyaluronan in respiratory system. In vivo stimulation with sHA caused increase expression of Marco receptor in WT and more exacerbated inflammation with increased level of cytokines and neutrophil influx in Marco-/- mice. Lack of Marco receptor causes less binding of sHA and balance baseline expression of TLR group proteins, which suggests a potential mechanism for this response.

1136 Comparison of Precision Cut Lung Slices and Whole Lungs in Particle-Induced Inflammation

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Precision cut lung slices (PCLS) have been widely used as a 3D organotypic lung tissue model to provide physiologically relevant responses to whole animal exposures while requiring few animals. We previously assessed lung toxicity of airborne particles in both PCLS and mice and sought here to determine whether the responses observed were linear between the two systems. The particles were categorized into three types: indoor airborne particles from an electronic recycling plant (mechanical process emissions; inorganic-rich particles; IRP), outdoor airborne particles (wildfire smoke; organic-rich particles; ORP), and engineered nanoparticles (SiO$_2$, CeO$_2$, and TiO$_2$; poorly soluble particles; PSP). PCLS were exposed to 22 µg/mL of IRP or ORP and 132 µg/mL of PSP under submerged conditions and assessed for lung toxicity at 24 h post-exposure. Mice were exposed to 100 µg of all the particle samples by oropharyngeal aspiration and assessed for lung toxicity at 4 and 24 h post-exposure. The relationship of proinflammatory cytokine levels (TNF-α, MIP-2, and IL-6) between PCLS and in vivo bronchoalveolar lavage fluid of mouse lungs was then examined. We found that ORP exposures show a strong correlation for all cytokines between the two models (TNF-α: r$^2$ = 0.9997, MIP-2: r$^2$ = 0.9953, and IL-6: r$^2$ = 0.9790), followed by IRP exposures (TNF-α: r$^2$ = 0.9957, MIP-2: r$^2$ = 0.7150, and IL-6: r$^2$ = 0.9390). PSP exposures however only showed a strong correlation for IL-6 (r$^2$ = 0.7948) between PCLS and whole lungs models (TNF-α: r$^2$ = 0.0075 and MIP-2: r$^2$ = 0.0605). These results suggest that inflammatory responses between PCLS and whole lung models are highly associated with concentrations of organic matter (ORP > IRP > PSP) and water-soluble materials (ORP > IRP > PSP) in the particular samples. In addition, IL-6 appears to be a more sensitive biomarker in PCLS than other cytokines. Overall our findings demonstrate good concordance in proinflammatory responses between PCLS and whole lung studies following exposures to different types of airborne particles. Notably, PCLS may better predict in vivo responses to chemicals (e.g., water-soluble organic matter) released from particles. This information can be used to understand the uses and limitations of PCLS as a screening tool for the lung toxicity of airborne particles. This abstract does not represent US EPA policy.
Extensive evidence indicates that exposure to ambient air fine particulate matter (PM) is associated with an increased risk of developing cardiovascular disease (CVD). Our previous studies have shown that PM exposure decreases circulating endothelial progenitor cell (EPC) levels in humans. In mice, exposure to concentrated PM (CAP) induces the depletion of circulating EPCs, decreases the function of ex vivo expanded bone marrow derived EPCs, and impairs EPC-mediated revascularization after hind limb ischemia. However, it is unclear whether PM exposure similarly affects cardiovascular processes in vivo. Hence, we tested whether exposure to CAP impairs angiogenesis by using matrigel implants. For this, male C57BL/6J mice were exposed to HEPA-filtered air or CAP for 4 days prior and 9 days after the matrigel implantation (500 μL containing 20 ng/mL VEGF, subcutaneous at the left dorsal posterior flank). CAP exposure decreased hemoglobin levels in the matrigel implants and the number of circulating EPCs (Fk-1+/Sca-1− cells). While CAP exposure decreased plasma nitrite levels, levels of plasma stem cell factor (SCF) or vascular endothelial growth factor (VEGF) were unchanged in plasma of CAP-exposed mice. Using the fluorescence reporter monochlorobimane (MCE), we observed increased permeability, suggesting that they result from the release of epithelial/fibroblasts (TEER) and small molecule (20kDa fluorescein isothiocyanate (FITC)-dextran) transporters. Upon DEP exposure, we identified a variety of oxidative stress responses in H441 cell monolayers of H441 cells, grown on the apical surface of Transwell permeable membranes, to the ubiquitous air pollutant, diesel exhaust particulates (DEP), with human lung fibroblasts on the underside of the Transwell membrane and human microvascular lung endothelial cells (HLEC) in the basal compartment. Upon DEP exposure, we identified a variety of oxidative stress responsive genes induced in the directly exposed H441 cells and in the indirectly exposed HLEC, including heme oxygenase 1 (HMOX1) and NADPH dehydrogenase (quinoxaline) 1 (NQO1). These changes in gene expression occurred despite a lack of change in trans-epithelial electrical resistance (TEER) and small molecule (20kDa fluorescein isothiocyanate (FITC)-dextran) permeability, suggesting that they result from the release of epithelial/fibroblast-derived mediators and/or compounds/metabolites that traverse the epithelial cells. Collectively, these data suggest that induction of oxidative stress in nearby endothelial cells may be mediated by epithelial cells, and may be an important mechanism of API-CVD. We conclude that we have developed a relevant, in vivo model of the AEB-cardiovascular system interface which can be used to identify the elusive molecular mechanisms driving API-CVD. Ultimately, data derived from this model can be used to identify therapeutic targets, biomarkers of both susceptibility and exposure effects, and can encourage the continued development of similar biomimetic models resulting in a reduction of non-essential animal testing.
1141 Pulmonary Fibroblasts Influence Epithelial Cells Response to Air Pollution

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The World Health Organization estimates that 8 million people die from air pollution every year. In addition to reducing the air pollution abundance and limiting the bad effects it is necessary to understand how inhaled chemicals affect tissues. Part of developing this understanding involves characterizing the relationship between air pollutants and their adverse effects, such as inflammation and oxidative stress, at the cellular level. While nearly every study to date has examined airway epithelial cells, the normal function of these cells is influenced by their interactions with other airway cell types as is referred to as the “airway epithelial microenvironment”. As a result, we hypothesize that fibroblast (IMR90) play a role in regulating the response of airway epithelial cells to air pollution exposures. We have developed an in vitro coculture model that allows us to determine whether the presence of airway fibroblasts, a key cell type in the airway microenvironment, alters the way that epithelial cells respond to air pollution exposure. 

1142 Cytotoxicity of Low Doses of Ultrafine Diesel Exhaust Particles in Endothelial and Microglial Cell Monocultures and Mixed Co-Culture

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Diesel exhaust particles (DEPs) are a recognized risk factor for several health conditions including neurodegenerative diseases. However, information regarding the toxicity of low doses of ultrafine DEPs in the brain is limited. While in vitro studies of cerebral capillary endothelial cell monocultures provide insight into the cellular mechanisms by which particulate matter (PM) cause toxicity, they do not account for the effects of cell-cell interactions on PM toxicity. The goals of this study were to evaluate the cytotoxicity of low doses of ultrafine (UF) DEPs and to investigate if cell-cell interactions mitigate or aggravate the effects of UF DEP exposure using rat brain microvascular endothelial cells (BMVECs) as a simple blood-brain barrier model and microglia as the model immune cells of the brain. Lactate dehydrogenase assay was used to determine cytotoxicity of endothelial and microglial monocultures, and in mixed co-culture exposed to UF DEPs for 24 hours. Results indicated that low doses of UF DEPs significantly decreased cell viability in a dose-dependent manner in the monocultures, but not in the mixed co-culture. Additionally, cell-cell interactions in the co-culture appear to have a protective effect compared to the microglial monoculture but not the endothelial monoculture. This study shows that exposure to low doses of UF DEPs can disrupt the function of BMVECs and microglia, and that cell-cell interactions can have the potential to mitigate cytotoxic effects from environmental toxins. Future studies involve repeating this experiment using a complete in vitro neurovascular unit model to further investigate the effect of cell-cell interactions on cytotoxicity of UF DEPs.

1143 Bronchial Epithelial Cells Exposure to Airborne Particulate Matter Results in the Expression of CIITA and an Increase of Bronchial Asthma Inflammatory Mediators


Inhalation of airborne particulate matter (PM) is associated with an increased risk of bronchial asthma in our population. PM composition includes low-toxicity minerals derived from natural and anthropogenic sources, and inorganic salts. Toxic metal levels are enriched in PM from polluted urban and industrial areas. Its organic constituents include polycyclic aromatic hydrocarbons (PAHs) which are considered to be widespread environmental contaminants and known for their carcinogenicity and mutagenicity. PM constituents are known to generate reactive oxidative species and thus alter gene expression. Furthermore, exposure to PM can induce respiratory and cardiovascular adverse effects by altering the available frequency, exposure and reactivity of lung antioxidant systems (ROS). Oxidative Stress in lung tissue caused by PM associated compounds correlates with an increase in asthma, airway fibrosis and COPD in urban populations. The exact mechanism describing bronchial epithelium (BE) exposure to PM and its contribution to inflammation remains unknown. Here we provide evidence of mRNA expression of the Major Histocompatibility Complex (MHC) class II and its major regulator, CIITA, in bronchial epithelial cells after exposure to organic PM extracts. Even though epithelial cells are non-professional antigen presenting cells (APC), bronchial epithelial cells express these pro-inflammatory mediators. Furthermore, induction of CIITA in BE results in an increased mRNA expression of pro-inflammatory mediators IL-6 and IL-8, characteristic of bronchial asthma. Our data thus provides evidence for an alternate inflammatory pathway upon exposure to airborne particulate matter in bronchial epithelial tissue.

1144 Afghanistan Particulate Matter Increases Mouse Airway Hyperresponsiveness Partially through IL-33 Signaling


An increasing number of soldiers returning from tours of duty in Afghanistan have reported asthma symptoms and other respiratory illnesses, possibly caused by inhaled nanoparticles from particulate matter (PM). However, it remains uncertain whether Afghanistan PM (APM) increases airway hyperresponsiveness (AHR), a key feature of airway diseases such as asthma. The IL-33 pathway, including its receptor ST2, has been implicated in the pathogenesis of lung diseases, but its role in PM-mediated airway dysfunction is uncertain. The goal of this work is to determine whether APM promotes AHR and inflammation in mice, and if the IL-33/ST2 axis is involved. Wild-type (WT) BALB/c and ST2 knockout (KO) mice (on a BALB/c background) were instilled with either a single dose of APM (1.25 or 2.5 mg/kg body weight) and euthanized 24 hours post-exposure. AHR was measured on live, anesthetized mice using the Flexivent animal physiology apparatus. Bronchoalveolar lavage fluid (BALF) and lung tissue were collected for analysis of inflammatory cells, cytokines, and gene expression. APM significantly increased AHR in WT mice, but not ST2 KO mice. Interestingly, both strains demonstrated significant increases in neutrophilic inflammation: 12-fold increase in WT mice and 3-fold in KO mice (p<0.0001 for all control vs. APM combinations). KC protein levels, a neutrophil chemoattractant, were significantly increased in BALF of WT and KO mice after the high dose of APM (p=0.01 and p=0.03, respectively). In WT mice, the high dose of APM significantly increased IL-33 mRNA expression in lung tissue (p=0.02), but not IL-33 protein in BALF. APM did not increase IL-33 protein levels in KO mice. APM increased inflammation in both WT and KO mice, but without ST2, there is no significant induction of AHR following APM exposure. The ST2/IL-33 axis may be essential for neutrophilic inflammation-mediated downstream events involved in AHR. Alternatively, AHR in the APM exposure model may be related to ST2-mediated tissue injury and repair processes, including mucus production or epithelial permeability.

1145 OH Formation from Fulvic Acid-Fe(II) Complexes in Human Lung Fluids

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Epidemiological studies show strong correlations between fine particulate matter (PM2.5) inhalation and increased mortality, cardiovascular and respiratory health effects. However, the biological mechanisms and PM2.5 components contributing to these health effects are poorly understood. A hypothesized mechanism contributing to these health effects is PM2.5 deposits in the lungs creating reactive oxygen species (ROS) and inducing oxidative stress. Out of all ROS we measure hydroxyl radicals (OH) due to its ability to oxidize lipids, proteins and DNA. Fe in the presence of lung antioxidants produces ROS, however the impact of Fe chelating lung fluid proteins (albumin and transferrin) on this ROS chemistry is poorly understood. It is accepted that albumin and transferrin can reduce ROS formation; however no studies measure OH formation from Fe(III)-albumin or Fe(III)-transferrin. Humic-like substances (HULIS) found in PM2.5 from biomass burning and cigarette smoke are capable of chelating with Fe(III). However the impacts of ROS formation in the presence of lung antioxidants is not fully understood. This study uses
Airborne fine particulate matter (PM2.5) derived from livestock farming is a unique mixture of dusts, biogenic materials and anthropogenic emissions. Susceptible individuals working in or living near high density animal operations may be at risk for adverse health effects associated with inhalation exposure to the ambient pollutants associated with these airsheds. To compare the potency of livestock farm-derived PM to exacerbate allergic airway responses in laboratory rodents, we collected PM2.5 from two chicken farms, two pig farms and two goat farms in the Netherlands. Female BALB/c mice (6-8 weeks old) were sensitized and boosted with ovalbumin (OVA; days 0, 10, respectively), and then challenged with intranasal saline or OVA for 2 consecutive days. OVA challenge was performed prior to a single intranasal exposure to OVA, 0.9 or 3 µg of farm-derived PM2.5. Twenty-four hours later mice were euthanized and bronchoalveolar lavage fluid (BALF) was collected for differential cell analysis and lung tissues were processed for light microscopy. All PM samples elicited significant, dose-dependent increases in BALF neutrophils in saline-challenged mice, with pig farm induced 2- to 6-fold greater inflammatory responses than other sources (rank potency goat PM >> pig PM > chicken PM). OVA sensitization and challenge induced allergic airway inflammation, as indicated by significant accumulation of inflammatory cells in BALF, mucous cell metaplasia in conducting airways, and alveolitis and bronchiolitis. In OVA-challenged mice, PM from both chicken and goat farms induced inflammation more potently than other sources (rank potency chicken PM >> pig PM > goat PM) compared to OVA challenged mice treated with saline. Both doses with 0.9 or 3 µg goat PM were equally potent. By comparison, treatment of OVA-challenged mice with PM from either of the chicken farms failed to alter the BALF inflammatory cell accumulations elicited by OVA. Lastly, PM from one of the pig farms produced a similar magnitude of airway responses as that elicited by the goat farm PM, whereas PM from the remaining pig farm induced non-significant changes similar to that induced by chicken farm PM. Our results suggest that the exacerbation of allergic airway inflammatory responses by livestock farm PM is dependent on the source of PM, and that physicochemical characterization of PM is needed to identify the specific components that might be responsible for the enhancement of allergic inflammation.

**Oxidative and Inflammatory Potential of PM2.5 from the San Joaquin Valley: Seasonal Trends and Molecular Marker Associations**

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Adverse health effects of ambient air particulate matter (PM) include respiratory and cardiovascular diseases, as well as cancer; contributing to an estimated 3.2 million premature deaths each year. Oxidative stress due to excess reactive oxygen species (ROS) production and inflammatory processes are hypothesized to be the main mechanisms underlying the adverse health effects associated with PM exposures. This study evaluated oxidative and inflammatory potential of the monthly PM2.5 compositions from a year-long sampling campaign conducted at two distinct locations in California’s San Joaquin Valley: Fresno and Bakersfield. The oxidative potential of the full dataset of PM2.5 samples was assessed using a broad-spectrum ROS probe (DCFH-DA), while the inflammatory potential was assessed via cytokine induction (TNFa by ELISA), both using an in vitro alveolar macrophage model. Furthermore, a subset of 0.9 µg PM composite samples from both sites was examined by RT-PCR for 28 different markers of inflammatory and oxidative signaling on the mRNA level. Relationships between PM-induced ROS and TNFa protein production, as well as PM chemical composition was examined using Pearson correlation. The chemical analysis of PM2.5 included organic carbon (OC), elemental carbon (EC), water soluble organic carbon (WSOC) and a large suite of organic molecular markers. A strong seasonal pattern was observed in both oxidative potential as well as cytokine production outcomes, with the latter having a more pronounced pattern at both sampling locations that correlated with PM chemical composition. Interestingly, no correlation has been found between the two measures of PM-induced toxicity suggesting that different chemical components drive each of these measures in the alveolar macrophage model. In addition, the mRNA cell signaling marker analysis revealed a distinct gene expression profile at each sampling site, with Fresno showing higher upregulation of the anti-inflammatory response markers.
in the NOR task 2 weeks before exposure ended and the OIP task on the last week of exposure. Mice were sacrificed for tissue harvesting the following week. Brain tissue was processed using standard techniques for immunofluorescent section staining, protein probing, and RNA analysis. UF PM exposed mice had decreased performance in both the NOR and OIP tasks compared to mice exposed to filtered air. Levels of amyloid deposition as measured by plaque burden were not affected, nor were amyloid peptide levels or APP gene expression. Conclusion: Behavior task results indicate that PM exposure can exacerbate memory impairment in AD model mice. However, this impairment does not appear to coincide with changes in amyloid pathology. These results suggest that alternative pathways leading to cognitive impairment, such as CNS inflammation state, are responsible for the decreased performance observed in this rodent model when exposed to ultra-fine particulate matter.

1150 Organosulfates Identified in Traffic-Related Air Pollution Are Neurotoxic in Primary Rat Hippocampal and Cortical Neuron-Glia Co-Cultures

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There is increasing evidence that traffic-related air pollution (TRAP) is an environmental risk factor for adverse neurodevelopmental outcomes, including neurodevelopmental disorders such as autism. Fine particulate matter (PM2.5) may especially contribute to neurologic disease. As defined by the United States Environmental Protection Agency, PM2.5 is comprised of several constituents including organic chemicals, metals, pollen and acids. Among the acidic atmospheric aerosols are organosulfates, which are estimated to comprise a significant fraction of PM2.5 mass. Organosulfates are abundant in TRAP in both rural and urban locations, but whether these compounds contribute to the neurotoxic effects of TRAP has not been addressed. The objective of this study was to screen organosulfates identified in PM2.5 for neurotoxicity in primary neuron-glia co-cultures dissociated from the hippocampus and neocortex of postnatal rats. Phenyl sulfate (PS), benzyl sulfate (BS), biphenyl 4-sulfate (9S), 3,4-nitrophenyl sulfate (4NS), 3-methylphosphoryl sulfate (3MS), glycolic acid sulfate (GAS) and hydroxyacetone sulfate (HS) were tested at concentrations ranging from 10 pM to 10 µM for effects on: (1) cell viability as measured by LDH release and uptake of calcein AM and propidium iodide; (2) neuronal morphogenesis determined by morphometric analyses of axonal outgrowth and dendritic arborization; (3) apoptosis measured by caspase-3 and -7 activities; and (4) mitochondrial function as measured by MTT. While none of the organosulfates altered cell viability, several were found to have biological activity that varied between hippocampal and cortical cultures. Significant increases in dendritic arborization were observed in cortical neurons exposed to 1 nM PS, 10 pM BS, or 10 µM HS; whereas, in hippocampal neurons, 100 nM HS, 1 nM GAS and 100 nM GS increased, but 100 nM 4NS decreased dendritic arborization. Apoptosis was decreased in hippocampal cell cultures by GAS at all concentrations, and in both hippocampal and cortical cell cultures by 10 µM BS. Overall, this study suggests that organosulfates may contribute to the neurotoxicity associated with TRAP exposures. Supported by NIH (P30 ES023513-03S1).

1153 Wastewater Reuse, Exposure Risk, and Fish Endocrine Disruption in the Shenandoah River Watershed

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Reuse of municipal wastewater treatment plant (WWTP) effluent is an important component in augmenting global freshwater supplies. One of the challenges associated with reuse of municipal wastewater is the presence of biologically active contaminants, such as endocrine-disrupting compounds (EDCs), which not only influence human consumption of re-used wastewater but also impact ecosystems which provide hydrologic connectivity between WWTP discharge locations and intakes for drinking water treatment plants. Risk assessment for potential endocrine disruption requires a landscape-based approach that allows investigations from river reach to continental scales. Widespread fish endocrine disruption, in the form of intersex and elevated plasma vitellogenin in male smallmouth bass, has been reported in the Shenandoah River Watershed (SRW). Detailed studies were conducted on the relationship between EDC exposure pathways and biological endocrine disruption effects incorporating wastewater reuse modeling, field sampling of water chemistry, and on-site fish exposure experiments. The amount of WWTP effluent in each river reach (calculated wastewater treatment plant discharge ratio - ACCWRatio) was determined and used to predict environmental concentrations for select WWTP derived EDCs, including the steroidal estrogens estrone, 17β-estradiol, estriol, and 17α-ethynylestradiol. Mobile laboratory fish exposure and water characterization experiments were conducted at 9 locations to assess different source waters for the presence of EDCs and fish endocrine disruption using vitellogenin induction in male fathead minnows as the biomarker. Although the various source waters had complex mixtures of chemicals, the measured and predicted environmental concentrations of select steroidal estrogens resulted in 17ß-estradiol equivalency quotients ranging from <0.5 to 5 ng L⁻¹, indicating low-to-moderate risk of fish endo-

1152 Wildlife Toxicity Assessment for 3-Nitro-1,2,4-Triazol-5-One (NTO)


3-Nitro-1,2,4-triazole-5-one (NTO) is a triazole and is comparatively less sensitive to toxic component that is used in many conventional formulations. NTO was developed recently as a potential replacement for energetic military munitions. The explosive nature of NTO was reported in 1985 and is now a key formulation component in the "insensitive munition explosive" (or IMX) series. When fielded, NTO use could be released to the environment and be found in soil, water and sediments at manufacturing facilities or training operations. We have reviewed acute and sub-chronic toxicity data of NTO in mammalian, avian, reptilian and amphibian species. Despite limited wildlife exposure data in animal studies, we have attempted to derive No Oberved Adverse Effect Level (NOAEL) and Low Observed Adverse Effect Level (LOAEL)-based Toxicity Reference Levels (TRVs) from dose-response end-points from target organ-specific studies. US Army Public Health Center Technical Guide 254 - Standard Practice for Wildlife Toxicity Reference Values (TRVs), guided our development of class-specific TRVs. Recently developed data is available for mammals and birds. By contrast, amphibian data are limited, and data relevant to reptiles are absent altogether. Tentative TRVs were derived from an oral sub-chronic study conducted in rats, and Benchmark Dose (BMD) values were derived from mammalian studies on exposure to NTO by ingestion. A BMD (ED10) of 70 mg/kg-day, and a lower-bound BMDL10 (LED10) value of 44 mg/kg-day was determined for the class Mammalia. For avian species, an ED10 for male quail was estimated at 62 mg/kg-day, based on brain lesions with a corresponding LED10 of 35 mg/kg-day. Using neuromuscular effects, an ED10 of 348 mg/kg-day was derived for male and female quail that corresponded to an LED10 of 151 mg/kg-day on oral exposure to NTO. For amphibian species, a NOAEC TRV of 334 mg/L and a LOAEC of 838 mg/L were derived following a 70-day exposure to NTO. These tentative TRVs are based on data from the most sensitive species available, and may be protective of the entire class of wildlife species with broad utility in ecological risk assessment applications.
was selected in order to assess the effects of mixtures of Oryzias latipes
1158 Effects of Low, Subchronic, Exposure of sperm from untreated adult zebrafish was incubated for 30 to 240 min with both experiments, sperm quality (after activation was observed for Erythromycin, followed by Propranolol. Sotalol was found to be affected for all mixtures, except two mixtures. The greatest main effect the scope of the particular study was to perform a chronic test with mixtures of APIs in order to evaluate some adverse effects towards an aquatic organism. The range of concentration was chosen based on the concentrations have been found in the environment mainly because they end up ubiquitously into the water cycle. Even at low concentrations, most of APIs can cause unrevealed toxicity to aquatic organisms. Usually, the behavior of APIs in a mixture may vary depending on the mixture composition, concentration and the chosen test to evaluate the results. It is well stated that even if a compound is not individually toxic, it may act in an antagonistic, additive or synergistic way with other drugs or metabolites, thus a toxic effect possibly takes place. The scope of the particular study was to perform a chronic test with mixtures of APIs in order to evaluate some adverse effects towards an aquatic organism. The range of concentration was chosen based on that populations of microorganisms are not exposed to pharmaceutically different mixtures, the immobilization percentage was found to be statistically different compared to the control. The total number of nauplii was found to be affected for all mixtures, except two mixtures. The greatest main effect was observed for Erythromycin, followed by Propranolol. Sotalol was found to have the least effect, followed by Diclofenac. The results are arguably realistic, based on that populations of microorganisms are not exposed to pharmaceutical mixtures in a fixed proportion.

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1157 Evaluation of Skin Mucus Vitellogenin (VTG) in a Japanese Medaka Fish Sexual Development Test (OECD TG 234) with 17a-ethynylestradiol (EE2) as Contribution to 3Rs Animal Welfare Concept
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Vitellogenin is regarded as sensitive biomarker to detect exposure to endocrine active chemicals in fish and provides mechanistic mode-of-action information. The current OECD Tgs 229, 234 and 240, recommend measuring VTG in the liver or head/tail homogenate of sacrificed fish. In the OECD TG 240 an extra cohort is used for whole body VTG measurement. VTG measurement in skin mucus of living fish is possible and may save individuals for other investigations. Implementing VTG measurement in skin mucus in an OECD TG 234 positive control study and comparison to established recommended procedures. The fish sexual development test was performed generally according to the OECD TG 234 with two replicates of 30 fertilized eggs each, in the chosen group and in the treatment group (0.1 µg/L EE2) in a flow-through system. Medaka fish (Oryzias latipes) were raised until day 60 post hatch. All recommended endpoints were measured. This poster focuses on secondary sex characteristics (stereomicroscopic investigation after modified Davidson’s fixation of the dorsal and anal fin), genotypic sex ratio (dmy gene with PCR), VTG levels in skin mucus and head/tail homogenate (ELISA) and histological evaluation of gonads, liver, and kidney in the midsection cut after fixation in Davidson’s solution. In this poster we present that the EE2 group all fish were phenotypically females as confirmed by histological investigation of the gonads. The test group and in the control group the phenotype and the genetic sex ratio in the EE2 group was not statistically different to the control. Female fish from the control and EE2 group could be differentiated from male controls by VTG levels in skin mucus as well as in head/tail homogenate. However, the coefficient of variation in each group was higher among the VTG in mucus compared to the head/tail VTG. Vitellogenin in skin mucus can be used as parameter especially for in interim measurements from living fish during the course of a study. However, the mucus sampling procedure should be further standardized to minimize variation and increase the power of this Parameter.

1156 Endocrine Disruptors Screening Analyzing Zebrafish Sperm Quality
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Endocrine disruptors (EDCs) are chemicals that by interfering with the endocrine system can have an adverse effect at developmental, neurological, immune and reproductive level. The negative impact of EDCs is becoming a real public health issue, therefore the necessity of tests to assess the potential risk of new chemicals before they are marketed is increasing. The zebrafish is currently used as a model for the evaluation of acute and developmental toxicity, and also for the screening and testing of potential endocrine disruptors as described in the OECD Guidelines. However, the two major endpoints used to evaluate endocrine disrupting chemicals, vitellogenin concentration and change in sex ratio, have several limitations. With the purpose of expanding the number of tests available to identify xenobiotics with endocrine disrupting activity, we developed an assay that evaluates the reproductive performance of zebrafish (after in vivo or in vitro exposure to EDCs) by measuring sperm quality using a computer-assisted sperm analysis (CASA). For in vivo experiments, adult zebrafish (aged between 6-12 months) were exposed to heavy metals (copper or mercury), herbicide (N-[phosphonomethyl] glycine) and bisphenol A for a period ranging 2-15 days. At the end of the exposure period the testes were excised, placed in tubes with immobilization solution and the sperm was released by gently shake. For in vitro experiments, sperm from untreated adult zebrafish was incubated for 30 to 240 min with four concentrations of copper, methyl mercury, N-[phosphonomethyl] glycin and bisphenol A. In both experiments, sperm quality (after activation with system water) was evaluated using a microscope with a negative contrast objective connected to a computer-assisted sperm analysis system (CASA) system (Proiser R + D., S.L., Paterna, Spain). Parameters such as progressive motility, curvilinear velocity and linearity were evaluated and compared to DMSO treated control. The assay here proposed with these two in vitro and in vivo approaches, can discriminate between compounds with a direct cytotoxic effect (reducing sperm motility) and compounds that might generate, after long exposure, a reduction in fertility by disrupting the overall process of spermatogenesis.

1154 Daphnia magna Chronic Toxicity Testing towards Using Widely Found Pharmaceuticals in the Aquatic Environment
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The rationale of the particular study was based on the fact that numerous mixtures of Active Pharmaceutical Ingredients (APIs) exist in the aquatic environment after wastewater treatment plants fail to reduce or eliminate them. APIs are considered as an enormous threat for the environment mainly because they end up ubiquitously into the water cycle. Even at low concentrations, most of APIs can cause unrevealed toxicity to aquatic organisms. Usually, the behavior of APIs in a mixture may vary depending on the mixture composition, concentration and the chosen test to evaluate the results. It is well stated that even if a compound is not individually toxic, it may act in an antagonistic, additive or synergistic way with other drugs or metabolites, thus a toxic effect possibly takes place. The scope of the particular study was to perform a chronic test with mixtures of APIs in order to evaluate some adverse effects towards an aquatic organism. The range of concentration was chosen based on that populations of microorganisms are not exposed to pharmaceutically different mixtures, the immobilization percentage was found to be statistically different compared to the control. The total number of nauplii was found to be affected for all mixtures, except two mixtures. The greatest main effect was observed for Erythromycin, followed by Propranolol. Sotalol was found to be affected for all mixtures, except two mixtures. The greatest main effect was observed for Erythromycin, followed by Propranolol. Sotalol was found to have the least effect, followed by Diclofenac. The results are arguably realistic, based on that populations of microorganisms are not exposed to pharmaceutical mixtures in a fixed proportion.

1158 Effects of Low, Subchronic, Exposure of Commercial 2,4-D Formulation on Early Life Stages of Native Wisconsin Game Fish Species
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Aquatic herbicides are used worldwide to eradicate nuisance and invasive plants despite limited knowledge of their toxicity to non-target organisms. 2,4-Dichlorophenoxyacetic acid (2,4-D) is a common active ingredient in commercial herbicide formulations, which triggers plant cell death by mimicking the plant specific hormone auxin. Application practices with 2,4-D commercial herbicides typically coincide with yearly freshwater fish spawning periods, exposing fish to xenobiotics at their vulnerable larval stages. However, the physiological impacts of 2,4-D on larval fish remain poorly understood, and hence, whether it may alter larval performance, fish populations, and ecosystem dynamics. We conducted a series of experiments to determine the effects of low concentrations (0.05, 0.50 and 2.00 ppm 2,4-D) of commercial amine salt herbicide formulation DMA®4IVM on the development and survival of nine freshwater fish species at various life stages. We observed reduced survival in embryo assays for 4 out of 9 species tested, reduced survival in 30-day larval assay for 4 out of 6 species tested, and no reduced survival in a 90-day juvenile assay in the 2 species tested. Altogether, the results indicate that the use of 2,4-D herbicide DMA®4IVM for weed control in aquatic ecosystems at current recommended concentrations (< 2 ppm whole lake; ≤ 4 ppm spot treatment) could reduce fitness and survival of freshwater game fish species.


**1159 Examination of the Avian Acute Oral versus Sub-Acute Dietary Testing in Ecological Risk Assessment**


The United States Environmental Protection Agency (US EPA), as well as other international regulatory agencies, require pesticide registrants to submit toxicity data that are used to conduct ecological risk assessments. While the US EPA has required both an acute oral and sub-acute dietary tests in birds, trends over the past 20 years have suggested that the avian sub-acute dietary test generally does not drive ecological risk assessment conclusions. To address this question, a retrospective analysis was conducted to evaluate 119 pesticides with publicly available ecological risk assessments that were registered into commerce between 1998 and 2017. New pesticides (i.e., registered in the US within the past 20 years) were chosen for the retrospective analysis to show utility of these tests for modern pesticide chemistries. Risk quotient (RQ) values (a point estimate of exposure divided by a deterministic toxicity endpoint) from the avian acute oral and dietary tests, as well as risk assessment conclusions, were compared to determine which test(s) drove the risk assessment findings. The RQ values were chosen as the data point for comparison in order to assess total risk (i.e., exposure and toxicity). After comparing RQ values from avian acute oral versus sub-acute dietary tests, there was only one case in which an avian sub-acute dietary RQ was greater than the acute oral RQ. In all other cases (greater than 99%), risks were not identified based on the results of both studies or the acute oral RQ was higher than the sub-acute dietary RQ. Based on the results of the retrospective analysis, it is concluded that in most cases avian risk can confidently be assessed without conducting the sub-acute dietary test.

**1160 Comparative Analysis of Potential Endocrine Effects of Triclosan in Three Model Fish Species and Xenopus laevis**


Triclosan (TS), a widely used antibacterial compound, was investigated for potential to interact with the endocrine system using US EPA and OECD test guidelines. A modified fish short-term reproduction assay (FSTRA) used three model fish species (Fathead Minnow (FM), Japanese Medaka (JM), and Zebrafish (ZF)), and the larval amphibian growth and development assay (LAGDA) used the amphibian model species, Xenopus laevis. All assays tested four concentrations along with a negative control. The FSTRA is used to identify compounds that may have potential to interfere with the normal structure and function of the hypothalamic-pituitary- gonadal axis. Thyroid histopathology was added to evaluate potential thyroid axis effects. In TS-exposed FM (average measured concentrations (amc) of 1.0, 2.7, 6.2, or 14 μg/L) at 14 μg/L mortality/morbidity was observed in males though not in females but fecundity was decreased. Histopathological findings (HF) in ovaries and testes at ≥6.2 and ≥2.7 μg/L, respectively, were not necessarily indicative of an endocrine system effect. In TS-exposed JM (amc of 4.7, 15.5, 57.5, or 179 μg/L) mortality was observed at 179 μg/L in both sexes. Decreased fecundity and ovarian HF were noted at 57.5 μg/L, suggesting possible endocrine system perturbation. In TS-exposed ZF (amc of 0.34, 1.3, 4.9, or 16 μg/L), in females, increases in standard length, gonadal weight, gonadal somatic index, and plasma vitellogenin were noted at ≥4.9 μg/L alone with decreased fecundity, increased body weight, and ovarian HF at ≥1.3 μg/L. Potential hormonome system disruption could not be dismissed. There were no thyroid gland HF attributed to TS in any of the species. ZF appeared to be the most sensitive to TS. The LAGDA is designed to evaluate apical effects of chronic chemical exposure on growth, thyroid-mediated amphibian metamorphosis and reproduction throughout multiple life stages. X. laevis embryos were exposed to TS (amc of 4.23, 13.8, 39.7, or 111 μg/L) until 10 weeks post-metamorphosis. Exposure to TS did not decrease survival, alter sex ratios or growth. However, median metamorphosis time was delayed at 111 μg/L. There were significant HF in gonadal ducts, testes, and kidneys predominantly at 39.7 and 111 μg/L, but not in ovaries, thyroid, and liver. TS effects in X. laevis and fish may support endocrine-mediated mechanisms that impair reproduction. This abstract does not necessarily represent US EPA policy.

**1161 Mitochondrial Dysfunction in Early-Staged Zebrafish Embryos Exposed to Chlorinated Persistent Organic Pollutants**


The exposure of chlorinated persistent organic pollutants (POPs) was known as one of important risk factors for metabolic syndrome (e.g., diabetes and insulin resistance), associated with impairment of mitochondrial function. An embryonic zebrafish has been applied into research on metabolic adverse effects, due to similar organ system and high genetic homology for disease with human. To investigate the effect of mitochondrial function on exposure of chlorinated POPs, we exposed individual zebrafish (i.e., 4.4'-DDT, Chlordane (technical mixture), Heptachlor, beta-HCH (be- ta-Hexachlorocyclohexane), Hexachlorobenzene (HCB)) and their mixture with 0.1% dimethyl sulfoxide (DMSO), to dechlorinated zebras fish embryos from 6 to 120 hpf (hours post-fertilization). We measured the oxygen consumption rate (OCR) and indirect indicators of the mitochondrial function, at sublethal concentrations of 0.05, 0.1, and 0.5 μg/mL, by using Seahorse XFe Extracellular Flux Analyzer at 24 hpf. In addition, we evaluated oxidative phosphorylation reaction rates on mitochondrial complex I to IV in isolated mitochondria, and analyzed mRNA expression of transcription factors (i.e., pparα, acox1, sda, acadm, and cs) regulating mitochondrial metabolism at 72 hpf. In our results, the OCR was reduced to the exposure of chlorodane and beta-HCH. In particular, OCR in zebrafish embryos exposed to the mixture decreased remarkably at lower concentrations. Dysregulation of oxidative phosphorylation in the mitochondria was identified on the complex I and III POPs, and the expression of sda, acadm, and cs genes were altered following exposure of p,p-DDT, chlordane, heptachlor and mixture. Consequently, the exposure of either individual and mixed chlorinated POPs impaired mitochondrial function biogenestically and bioenergetically.


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**1162 Toxicity Study of Perfluorohexanoic Acid (PFHxA) and Its Salts in Aquatic Animals**

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Perfluorohexanoic acid (PFHxA) belongs to polyfluoroalkyl substances (PFAS). It is an environmental degradation product of C6-based fluorotelomer intermediates, used to produce various kinds of polymers. PFHxA is considered as a low toxic chemical compared to PFAS. Therefore, PFHxA is used as a replacement for long chain PFAS in industry site. However, the concerns of its ecotoxicity still remain. Therefore, we performed exposure experiments by using two kinds of aquatic animals, medaka (Oryzias latipes), rainbow trout (Oncorhynchus mykiss). To investigate the potential effects to fish reproduction of perfluorohexanoic acid, ammonium salt, we first performed experiment by using medaka (Oryzias latipes). We found that NOEC of PFHxA-NH4 and PFHxA-Na for the reproduction of medaka were both above100 μg/L. The spawning in the exposure levels was active the same as control, and decreasing trend was not observed compared with the control. Although the fertility tended to decrease in 100 μg/L PFHxA-NH4 and PFHxA-Na exposure levels, it kept more than 90% and there was no significant difference. Furthermore, VTG production was not detected in any male fish. Therefore, it was indicated that PFHxA-NH4 or PFHxA-Na has no estrogenic activity. Next, we examined the effect of APF on growth and development of embryos and larvae of the freshwater fish species, rainbow trout (Oncorhynchus mykiss), in a Fish Early-Life Stage Toxicity Test (ELS). Hatching success in the control group was 74%. As this exceeded 66%, the validity criterion for hatching success was satisfied. First egg hatch in treatment and control vessels occurred in the 24-hour period between the Day 25 and Day 26 pre-hatch observation timepoints. This indicated no difference in time to first hatch across all treatments when compared to the control group. Larval survival until Day 28 post-hatch in the control group exceeded 70% (93%) thereby satisfying the validity criterion for hatching success. Post-hatch survival, average daily weight gain, and percentage of fish that grew ranged between 96 and 100%. Additionally, exposure groups did not show significant difference with control group in both fish total lengths and wet weights. The NOEC and LOEC for hatching success, larval survival, fish total lengths and wet weights are considered to be 9.96 and over 9.96 mg/L respectively. The NOEC and LOEC for post-hatch larval survival until Day 28 are 2.96 and considered to be low. In conclusion, PFHxA and its salt have no estrogenic activity and no effect on reproduction to aquatic animals in the test concentration.
Silicones—Do They All Behave the Same Way in the Environment Regarding Biodegradability/ Degradability?

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Many molecules are found under the generic name of silicones. Applications are numerous and silicones are found in industrial, pharmaceutical, medical device and personal care products. Revisiting the concept of biodegradability and degradability in such a complex family is challenging. Biodegradability was investigated using in silico tools (Epiwin and QSAR toolbox) and it was concluded that silicon-based molecules are hardly handled with these softwares. From our literature survey and our experience, we recommend that a non-conventional approach can be taken with the aim of studying potential relevant effects such as a solar simulator to study UV effects combined with abiotic degradation in wet and dry soils. Physico-chemical properties, in part driven by the polymerisation level, play a crucial role as they influence the physical state: solid, liquid, volatile, gel. Functionalisation of alkyl dimethicone compounds is also a key parameter for both entry in the environment and degradation. Silicones in cosmetics should be considered separately because of particular conditions of use of these products and the fact that they are found in wastewater in which it is treated in consequences. Abiotic processes can be important steps in the degradation of man-made chemicals in the environment (clay acts as a catalyst of degradation of silicones); usually only primary degradation occurs but the products formed may be biodegraded further by microorganisms. The ultimate stage, represented by the siloxane backbone degradation, may be obtained by the combined effect of bacteria, clay and UV light leading to formation of silicates. A low level of polymerisation leads to easily hydrolysed silicones by enzymatic and non-enzymatic reactions of the grafted carbon chain. A higher level of polymerisation provides greater protection against degradation. Consequently (bio)degradability should be approached on a case-by-case basis and the approach should consider parameters which are specific to silicones. Even if (bio)degradation, with respect to the OECD definition for very high molecular weight silicones, is not totally achieved, a degradation of lower molecular weight silicones, as used in the personal care industry, is achieved by other means such as UV and abiotic transformation.

Reconstituted Mining Effluent Reduces Neuronal Proliferation in the Developing Brain and Slows Growth of Body and Facial Features in Wild-Caught Wood Frog Tadpoles


Mining has been a dominant industry in rural Appalachia for more than 150 years, but the impact of mining on aquatic animal health is not well understood. This is an important issue because Appalachia is home to an enormous diversity of organisms, including a huge array of amphibians that live in streams that receive mining effluent from operating and abandoned mines. We examined the effects of reconstituted mining effluent on the development of wood frog (Lithobates sylvaticus) tadpoles. We collected day-old fertilized eggs from a creek near Blacksburg, VA in early March, 2018 and raised them to hatch. Tadpoles were then assigned to either sulfate or chloride-based reconstituted mining effluent diluted to six different conductivities (100 µS/cm - 2,400 µS/cm). After 7 or 14 days of treatment, tadpoles were euthanized and fixed in paraformaldehyde. We imaged the heads and bodies of tadpoles for morphometric analysis before dissecting out brains and immunostaining them for phospho-histone3, which labels dividing progenitor cells in the brain. We found that sulfate-based reconstituted mining effluent significantly lowered progenitor cell division at 1200 µS/cm at Day 7 and at 600 µS/cm at Day 14 relative to control. Chloride-based reconstituted mining effluent was less impactful, with no significant differences observed at Day 7 and significantly lowered progenitor cell division at 2400 µS/cm at Day 14. In addition, both treatments slowed growth of morphological features, including body length, head size, and interocular distance. In contrast to the neuronal data, higher concentrations of effluent appeared to increase variability within each treatment. These experiments suggest that mining effluent that has high concentrations of sulfate is particularly likely to negatively impact the development of amphibians.

PBDE-47 Can Affect the Embryonic-Larval Development of Zebrafish but Not BDE-99 and BDE-154

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Polybrominated diphenyl ethers (PBDEs) are widely used as flame retardants that have been detected in samples of humans and wildlife animals. Their physical and chemical properties favor their bioaccumulation and provide high resistance to environmental degradation. The congeners BDE-47, BDE-99 and BDE-154 are between the most used and present high toxic potential, such as induction of endocrine disruption, neurotoxicity and hepatotoxicity to humans. It is known that PBDEs can be accumulated in the environment, especially in aquatic organisms, thus we evaluated the toxicity of these compounds in an ecotoxicological model. Zebrafish (Danio rerio) was used to evaluate the toxicity of these environmental contaminants on its embryonic and larval stages. Endpoints of lethality (e.g. coagulation, hatchling), sub-lethality (e.g. eye development, pericardium and yolk edemas) and teratogeneticity (e.g. delayed growth, inflated swimming bladder) were assessed after embryo exposure to BDE-47, BDE-99 and BDE-154. The highest tested concentration of BDE-47 (12.1 mg/L) was able to cause toxic effects, such as pericardium and yolk edema induction, however most of them were reabsorbed until 144 hours post fertilization (hpf), thus not culminating in lethal effects on zebrafish embryos/larvae. Teratogenetic effects have been observed to those BDEs, but we did not significantly detect in zebrafish embryos/larvae after BDE-47, BDE-99 and BDE-154 short-term exposure. Therefore, the exposure of zebrafish to BDE-47, but not BDE-99 and BDE-154, can impair zebrafish development with slight toxic effects.

Developmental Exposure to Fluoxetine Induces Higher Mortality but Not Gross Anomalies in an In Ovo Chicken Model

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Fluoxetine is a pharmaceutical pollutant of emerging concern as it is detected in trace amounts in myriad water sources. Several studies have demonstrated that environmentally relevant concentrations of fluoxetine impact behavior, reproduction, and survival of aquatic species. Fluoxetine has been shown to impact the stress axis in small aquatic animals and plants at concentrations similar to those found in the environment. It is well known that developmental and early life stress from environmental contaminants profoundly affect physiological regulation throughout life. Previously, we investigated phenotypic anchors associated with modulation of the stress axis by fluoxetine in an avian model of developmental toxicity. We found that fluoxetine induced molecular changes in the stress-axis of newly-hatched chickens exposed in ovo. These data led us to hypothesize that developmental changes induced by fluoxetine exposure will persist and lead to detectable effects in two-week old chickens. Prior to incubation, fertilized chicken eggs were injected with environmentally relevant concentrations of 0, 1, 10, or 100 ng/mL of fluoxetine in DMEM. Eggs were incubated until hatch and hatchlings were kept until 13 days of age. Hatchlings were monitored for growth throughout the study and tissues were collected and weighed after necropsy. Body and organ weights, and general growth, as measured by increases in body weight and crown-rump length, did not differ among groups. However, all treated groups exhibited at least 5% higher embryonic mortality than vehicle controls. Together, our findings indicate that developmental exposure to fluoxetine can affect survival and induce molecular changes relevant to the stress axis, but does not impact less-sensitive endpoints, such as growth. Because developmental impacts to the stress axis are often not apparent until a secondary insult, our additional work investigated how individuals responded to an inflammatory challenge. Here, we assessed for an inflammatory response using a PHA skin test, which showed a dampened response in animals treated with the lowest concentration of fluoxetine, and increased variability in those treated with the highest. Further analyses will determine relationships imperative to the stress axis, including glucocorticoid receptor concentrations, corticosterone level and other immune function parameters, that could help explain pollutant-induced mortality.
1167 Monitoring Reproductive and Immunological Effects in Colonial Waterbirds to Support Management Decisions at Contaminated Great Lakes Sites


This monitoring program assessed effects of contaminants, primarily PCBs and PCDDs, on immune function and reproduction in fish-eating birds in the Saginaw Bay and River Raisin Areas of Concern (AOCs) and Grand Traverse Bay in 2010-18 under the Great Lakes Restoration Initiative and AOC programs of the US Fish and Wildlife Service and US Environmental Protection Agency. Saginaw Bay sites included two herring gull colonies (Confined Disposal Facility (CDF) and Little Charity Island), two Cassip tern colonies (CDF and Charity Reef/L. Charity Island) and one black-crowned night heron colony (CDF). Herring gulls were studied in the River Raisin AOC at the Detroit Edison Monroe Power Plant on the western shore of Lake Erie and on Bellow Island in Grand Traverse Bay. Reference sites were in the lower St. Mary’s River (gulls on Pipe Island Twins and terns on Two Tree Island), on Tahquamenon Island in Whitefish Bay (terns), and on Chantry Island, Lake Huron (herons). Gull embryos were assessed during late incubation using a viability detector sensitive to heartbeat and movement. Relative risk ratios for embryonic nonviability were significantly elevated two to three-fold at contaminated sites compared to the reference site (2.2 for the Saginaw Bay AOC, 2.7 for the River Raisin AOC, and 3.4 for Grand Traverse Bay). Infertility was the primary cause of nonviability at the reference site. Elevated infertility and mortality contributed to nonviability in contaminated sites. Deformities associated with PCBs and PCDDs were found in several individuals at contaminated sites (3 gull chicks on Monroe, 2 tern chicks on L. Charity, 3 gull embryos on the CDF, 1 gull embryo on L. Charity, and 1 gull embryo on Bellow). Chick productivity in terms of Saginaw Bay (mean of 0.76 chicks/nest) was significantly below that of reference sites (1.2 chicks/next). In the River Raisin AOC, productivity of gull chicks was poor for 4 of 9 years, with complete reproductive failure in 2010. In gull chicks the mean phynohemagglutinin (PHA) skin response for T-cell mediated immunity was suppressed 54-56% at both AOCs and 50% in Grand Traverse Bay. This response was suppressed 48% in terns and 39% in herons in Saginaw Bay. Mean antibody responses in gull chicks at the River Raisin AOC and in Grand Traverse Bay were two- three-fold lower than at the reference site. Ongoing immunological and reproductive impairments at these contaminated sites are consistent with the effects of persistent pollutants such as PCBs and PCDDs.

1168 Tebuthiuron and Trifluralin Affect Locomotor Activity of Zebrafish (Danio rerio) Embryos and Larvae Body Length

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Tebuthiuron (TFL) and tebuthiuron (TBR) are herbicides commonly applied in sugarcane culture leading to environmental occurrence, as reported by several studies. However, little is known about TFL and TBR toxic potential on non-target organisms. In this study, we analyzed effects of TFL and TBR exposure on embryo locomotor activity and larvae body length of zebrafish (Danio rerio). For this purpose, five concentrations of TFL and TBR analytical standards (0.1, 1, 10, 100, and 1000 µg/L) were tested. Dimethyl sulfoxide (DMSO) 0.01% was used as solvent control. During exposure, embryos and larvae were kept at 28 ± 0.5°C with constant light-dark (14:10h) cycle (only for TBR treatment group but not for TFL). Within 27 to 30 hours post-fertilization (hpf), embryos locomotor activity (n=90) was monitored for 3 minutes. Data were analyzed by DanioScopeTM (Noldus) software, quantifying percentage of burst activity, burst count, mean burst duration and total burst duration. Larvae body length (n=60) was measured at 96 hpf. Analysis of variance (ANOVA) followed by Dunnett’s Multiple Comparison Test (p <0.05) was performed by SPSS Statistics (IBM). Results showed that TFL at highest concentration (1000 µg/L) has decreased larvae body length in comparison to control group (F3,564 = 42.95, p<0.001), but did not affect embryos locomotor activity. On the other hand, TBR strongly decreased embryos locomotor activity inducing less locomotor activity even at lowest concentration tested (0.1 µg/L). Additionally, decreasing of locomotor activity was dependent on the concentration tested (highest concentration led to lowest locomotor activity). Our findings allow us to evidence TBR ability to interact with biological organisms such as zebrafish at early life stages, as well TFL capacity to induce teratogenic effects.

1169 Yeast Atlas, Diversity of Wild Yeast Collected from North and South American Regions

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The ecology of wild strains of Saccharomyces cerevisiae from around the globe is poorly understood. Early domestication by human kind and ubiquitous use of a select few strains has led to an ambiguous origin, although relatively new data suggests an out of Asia theory and importance of insect vectors in yeast mobility. Glyphosate is an herbicide which is commercially used as the main ingredient in Roundup®. Prolonged use has increased glyphosate resistant plants, which may affect human health. In this study, urban and rural isolates were obtained through phenol-chloroform extraction of genomic DNA and amplification of their internal transcribed spacer (ITS) gene of the ribosomal DNA. Sanger sequencing was employed and used in conjunction with NCBI databases for identification of yeast genus. Using species specific multiplexing primers, species level identification has been resolved. Development of The Yeast Atlas as resource to better understand mechanisms of response, adaptation, and evolution to stimulants such as chemicals including glyphosate is a tool for yeast researchers to harness the genetic diversity of wild strains with ease. This effort is currently on going: genetically diverse wild yeast are being both processed and identified. Once identified, the collected S. cerevisiae samples will be categorized by location and evaluated for phenotypic responses to chemicals. Greater than 623 samples have been amassed and 538 isolates recorded. PCR amplification of 421 amplicons aided in 329 species being matched in the NCBI database. Phylogenetic diversity of the isolates varies to include Pichia, Lanchancea, Candia, and more families of yeast other than Saccharomyces. North and South American isolates of S. cerevisiae will be assayed against chemical stressors, including glyphosate, as a screening process for phenotypic traits of wild yeast.

1170 An Overview of the Properties MCHM Exhibits on Multiple Yeast Strains

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In 2014 a chemical spill occurred in the Elk River, West Virginia releasing approximately 10,000 gallons of the coal washing chemical, 4-MCHM (NIIH, 2015). The contaminated water affected aquatic organisms, local plant and animal species, and nearly 300,000 residents residing in the Charleston, WV area (Pusey and West, 2015). In this study we had selected multiple yeast strains that were grown in constant exposure to MCHM. Our goal is to develop yeast strains resistant to MCHM and analyze their DNA sequences to detect specific mutations at perspective genes. Serial dilutions on solid as sayso presented numerous strains presenting resistant-like properties. Further genetic analysis revealed similarities in the polymorphisms between some of the resistant strains. The mutations range from non-synonymous point mutations to addition/deletion frameshift mutations ~40 base pairs long. Similarly, on numerous occasions the same mutations occurred between two parallel strains. MCHM may cause DNA replicative stress. Mechanisms of possible DNA damage and the promotion of protein aggregation are a few of the leads thus far. Further analysis of the mutations will guide our understanding to the toxicological effects of MCHM.

1171 Analysis of Emerging Contaminants in Maryland Coastal Bays Using In Vitro Bioassays as Biological Screening Tools

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Emerging contaminants (ECs) with estrogenic or estrogen-like activity have been increasingly detected in aquatic environments and have been an issue of global concern due to their potential adverse effects on wildlife and human health. ECs include pharmaceuticals, personal care products, natural and synthetic hormones, nanomaterials, food additive, plasticizers. These chemicals and their bioactive metabolites are usually introduced to the aquatic environment as a complex mixture through wastewater treatment systems. This study was aimed to investigate the fate and occurrence of ECs in the Maryland Coastal Bays (MCB) and to assess their estrogenic activity profile. Fish samples were collected from four sites as well as water and sediment samples were collected from 13 sites along the MCBs in addition to influx and effluent samples from Ocean Pines and Princess Anne Wastewater Treatment Plants (WWTPs). In this study, Stripped killifish (Fundulus majalis), and Mummichog (Fundulus heteroclitus) were used as bioindicators for spatial contamination. Samples were analyzed using vitellogenin (VTG) assays as biomarker for the presence of ECs in the environment and MCF-7 cell prolifer-
1172 Mapping Nutrient and Heavy Metal Concentrations in Greens Bayou

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Houston is the fourth largest and one of the fastest growing cities in the United States. The rapid population growth of the greater Houston area has caused major urbanization to its local watersheds. The Greens Bayou Watershed is located in north Harris County and encompasses portions of the cities of Houston and Humble. The watershed covers about 341 kilometers (212 miles) across the city. One of the main waterways connected to the watershed is Greens Bayou. As the population increase in the greater Houston area, the heavy metal waste and pollution increases. Waste runoff is also growing rapidly in the city of Houston. During September of the year 2017, a major hurricane named Hurricane Harvey flooded the city of Houston. The goal of this study is to monitor the changes in the nutrient and metal concentrations in the water and flood plain soils of Green Bayou during the pre- and post-Harvey event. Water and soil samples were collected from Greens Bayou during the pre- and fall of 2017. The instruments used to identify the nutrient and metal concentrations in the water and soil samples were total organic carbon and nitrogen (TOC), inductively coupled plasma mass spectrometry (ICP-MS), and the hand held X-ray fluorescence analyzer (XRF). Our water sample analysis indicates that the TC, TN, P, K, Ca, Na and Mg is significantly higher in the upstream locations compared to the downstream locations during the pre-Harvey sampling. On contrast to the other elements, Fe concentration increased significantly in the downstream locations compared to the upstream locations during pre-Harvey sampling. The N, P, Ca, Na, Mg, Fe and Zn concentrations in the downstream locations increased following the Harvey flooding. The total N and total P concentrations in our water samples as well as the historical data exceeds the US EPA nutrient criteria for rivers and streams which is 0.76 ppm for total N and 0.128 ppm for total P. All the metal concentrations in the water were found to be below the critical limit while the N and P concentrations exceeds the critical limit. Future research involves identifying the non-point sources of contaminant, which can be better managed to preserve the health of the urban watershed ecosystem.

1173 Culture System Differences in Growth and Hematological Profiles of Juveniles and Adults Clarias gariepinus

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Clarias gariepinus, a typical freshwater clariid species in the coast of Africa is an essential fish species given its immense contribution to the nutritional needs, economic growth and development of any African nations including Nigeria. Knowledge of current health status of Clarias gariepinus reared in different culture media is lacking. Therefore, this study was conducted in six randomly selected fish farms (concrete tanks and earthen ponds) in three agricultural zones in Lagos State, South Western Nigeria. Growth and blood indices for hematological variation in the upstream locations of Greens Bayou were compared to the downstream locations during the pre-Harvey sampling. The results showed that water quality characteristics of earthen ponds recorded significantly (p < 0.05) higher level of ammonia concentrations, lower values of dissolved oxygen, pH and transparency than in concrete tanks indicating poor water quality. Haematological indices: red blood cells, packed cell volume and hemoglobin increased significantly (p < 0.05) in fish reared in concrete tanks with highest mean red blood cells and packed cell volume and hemoglobin increased significantly (p < 0.05) in fish reared in earthen ponds are diagnostic elements, predisposing factors for microcytic fish anemia, a health phenomenon in cultured catfish in Nigeria.

1174 An ‘Omics Approach to Unraveling the Paradoxical Effect of Diet on PFOA- and PFNA-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 role of environmental exposures as risk factors for fatty liver disease is not well known. Perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFNA) are widespread environmental toxicants that persist in over 98% of the general population. The aim of this study was to evaluate whether PFOS or PFNA exposure in combination with a moderately high-fat diet, augmented hepatic lipid content and biomarkers associated with NAFLD. Six-week-old male C57BL/6 mice were fed either a 10% kcal low-fat diet (LFD) or 45% kcal high-fat diet (HFD), with or without 0.0003% PFOS or PFNA (LFD-PFOS, LFD-PFNA, HFD-PFOS, and HFD-PFNA, respectively) for twelve weeks. The HFD increased liver weight by about 30% and body weight compared to the LF control. Both HFD-PFOS and HFD-PFNA administration significantly increased liver and body weights when compared to the LF control. PFNA induced significant liver weight increase over both the control groups, as well as PFOS-exposed mice. Internal hepatic PFOS and PFNA content was quantified by LC-MS and the impact of diet on hepatic tissue was assessed through various histological and H&E techniques. PFOS and PFNA were compared and the additional impact of diet on these mechanisms was assessed. Both PFOS and PFNA treatment resulted in significantly increased expression of fatty acid uptake genes cluster of differentiation 36 (CD36) and solute carrier family 27 member 1 (Slc27a1), while PFOS-exposed mice showed an opposite effect. Both PFOS and PFNA exposure resulted in more profound effects on gene and protein expression overall when compared to PFOS. Diet exerted an additional impact on the mechanisms and potency of PFOS and PFNA in the liver. The data suggests that PFOS and PFNA at an exposure relevant dose (0.0003%) may have an adverse effect on hepatic lipid accumulation when combined with a LFD. The paradoxical impact of diet on the mechanism and potency of PFOS and PFNA is described herein.

1175 Comparative Analysis of International and Domestic Points of Departure and Uncertainty Factors Contributing to Disparate Oral Reference Doses for PFOA

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Perfluorinated aliphatic carboxylic and sulfonic acids have been widely used in consumer and industrial products, such as firefighting foams, surfactants, chemical treatments for stain resistance, and key components for the manufacture of fluorinated polymeric materials. Since the 1950s, this category of compounds contain very stable C-F bonds, making them resistant to metabolism, phototransformation, biodegradation, and hydrolysis, with a human half-life of approximately 3.8 years. One of the most prevalent perfluoroalkyls is perfluorooctanoic acid (PFOA). PFOA has been detected in various environmental matrices (e.g., soil, surface and ground water, air, and food) and is biopersistent. As such, PFOA has been widely scrutinized for regulatory oversight and several agencies have established screening values for use in risk assessment of PFOA. This work presents a comparative analysis of International and US federal- and state-level recommended oral reference doses (RfDs) and the underlying variance in the approaches used to determine these RfDs, including selected points of departure (POD), treatment of pharmacokinetics, and uncertainty factors (UFs). Currently, international and domestic regulatory agencies' RfDs for PFOA range from 0.0015-0.2 µg/kg/d and are based on varying UF s, PODs, species, and endpoints. These values are based on human equivalent dose (HED) pharmacokinetic models that use lowest observed adverse effect level (LOAEL), no observed adverse ef-
flect level (NOAEL), and/or BMDL10 (benchmark dose level associated with 10% risk of adverse effect in exposed test animals). We have also performed rigorous study selection analysis of the 20+ studies relied upon for RfD calculations. From these analyses, we have found that more conservative RfD values are primarily due to post-study selection treatment of UFs and use of LOAEL as POD. Interestingly, some of the more conservative RfD values have a narrower exposure range for common organisms (e.g., mice, rats, monkeys) and critical effects (hepatotoxicity, developmental toxicity, and reproductive effects), suggesting significant inconsistencies between various regulatory agencies. The heterogeneity of international and domestic recommended RfDs will continue to be a significant issue for future PFOA regulatory action.

1176 Approaches for Assessing Perfluoralkyl Acid Mixture Toxicity: A Case Study with PFOA and PFHxS

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The relative potency factor (RPF) method is one approach for addressing mixtures that focuses on deriving a set of toxicity values that are normalized to a reference compound. This approach has been used to assess the toxicity of complex mixtures of PCBs and dioxin-like compounds that share the same mode of action (MoA) for eliciting an adverse effect. Interest in RPF methods for perfluorinated alkyl substances (PFASs) is growing given developments from in vitro studies and non-mammalian models in understanding potential MoAs of PFASs, both individually and as mixtures. Criteria for assessing whether or not the RPF method is applicable to specific PFASs include: 1) identifying if responses are mediated through a single receptor; 2) evaluating species differences in receptor responsiveness; 3) determining if differences in dose-response are consistent across relevant dose ranges. In this presentation, we demonstrate that PFASs do not satisfy these criteria by comparing perfluorooctanoic acid (PFOA) and perfluorohexanoic acid (PFHxS), which differ in structure by only two fluorinated carbons. Studies demonstrate that, unlike PFOA, PFHxS is a weak carcinogen, hepatotoxicant, developmental or repro- ductive toxicant, or an endocrine disruptor. The only human-relevant adverse effect reported for PFHxS is histopathological changes in the kidney of female rats in a 2-year study, which yields NOAEL and LOAEL values of 30 and 200 mg/kg-day, respectively. This LOAEL is 200-fold higher (i.e., less toxic) than reported LOAELs for PFOA (i.e., approximately 1 mg/kg-day) in mice. Low levels of PFHxS in the kidney may be due to lower dose, differences in receptor responsiveness, or other factors. Analysis of high-throughput Tox21 data demonstrates that both PFOA and PFHxS are PPARα agonists, however, unlike PFHxS, PFOA exhibits binding to additional nuclear receptors, which may contribute to its MoA. Quantitative dose-response relationships for PFASs and PFHxS do not exhibit consistent proportional differences in response across relevant dose ranges. The RPF approach is contrasted with the conventional hazard index method to demonstrate how these approaches, if applied in a risk assessment context, may support very different risk management decisions depending on exposure ranges, differences in human relevancy of endpoint selection, and different potency factors applied in deriving mixture toxicity criteria for reference doses. Collectively, these results demonstrate that even highly structurally similar PFASs may not satisfy minimum criteria for the RPF method.

1177 PFOA and PFOS Interfere with Hepatocyte Differentiation in a Human iPSC to Hepatocyte Model

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Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) are persistent organic pollutants with significant concerns for health of humans and the environment. PFOA/OS cross the placental barrier and accumulate in fetal liver. In utero exposure to these compounds is associated with decreased birth weights in humans. We hypothesized that PFOA/OS exposure inhibits hepatocyte differentiation during embryonic liver development. To test this, we used a human induced pluripotent stem cell (iPSC)-derived hepatocyte model, which recapitulates various stages of embryonic hepatocyte differentiation in vitro. An iPSC line (K3) was differentiated to hepatocytes using a culturing protocol that directs differentiation of the cells into definitive endoderm (Day 0 - 5), specified hepatic endoderm (Day 6 - 10), immature hepatocytes (Day 11 - 15), and mature hepatocytes (Day 16 - 20). Gene expression analysis confirmed activation of mature hepatocyte markers (AFP, ALB, CYP2C19, F7, GSTA1, HNF4A, TP) and downregulation of developmental and stem cell markers (CXC4, GATA4, SOX17) following completion of the differentiation protocol. Next, 10 μM PFOA, PFOS, or vehicle was added to culture media from days 6, 11 or 16 of iPSC to hepatocyte differentiation and samples were obtained at day 20. PFOA treatment was more effective than PFOA in disrupting the iPSC to hepatocyte differentiation. Treatment from days 16 to 20, which correlates with immature to mature hepatocyte differentiation was the most affected stage by both PFOA and PFOS. Day 6-20 treatment of PFOA resulted in reduced CXC4 expression and sustained expression of SOX17 expression. Day 16-20 PFOA treatment enhanced CYP2C19 expression but blocked expression of CXC4. PFOA treatment upregulated hepatocyte cell markers (CXC4, SOX17) and also reduced expression of hepatocyte markers (ALB, CYP3A4, F7, HNF4A). In conclusion, these data indicate that PFOA and PFOS have significant effect on embryonic hepatocyte maturation and that this iPSC to hepatocyte model of in vitro hepatic differentiation is an effective method to identify the underlying mechanisms.

1178 Concentration of 19 Perfluorinated Compounds in Processed Seafood Analyzed by Liquid Chromatography-Tandem Mass Spectrometry

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Perfluorinated compounds (PFCs) that are kind of anthropogenic contamination materials existed in ubiquitous environments such as water, soil, air, and foods. PFCs are accrued in the human body and hard to discharge. Recently, there were studies about monitoring PFCs in seafood. In this study, 19 PFCs were monitored by in-house developed liquid-liquid extraction methods after homogenizing samples and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The validation of our analysis method was performed by accuracies and precisions using inter-day and intra-day. The accuracies were between 80% and 120% and the precisions were within 20%. The limit of quantitation (LOQ) of 19 PFCs was ranged from 0.04 to 0.18 ng/g. Our samples were composed of 302 processed seafood that was directly in consumption form to the consumer. These samples were divided 5 categories such as processed fish products, dried seafood, salt seafood, canned food, and seasoned laver. In this study, the results showed that PFOA was the highest concentrated in processed seafood (40-100 ng/g) in canned food and salt seafood, PFOA in dried seafood, and PFPeA in seasoned laver. Although there were the different patterns of the highest PFCs in each category, PFOA and PFOS were mainly detected in all categories. The dried seafood (including seasoned laver) and salt food were detected a high level of PFOA and PFOS compared with other categories. Also, the present study show that the distribution of the PFCs’ concentration were different as per biological classification, the raw material of samples, and habitat of the raw fish samples using statistical analysis. The result was that crustaceae, sea squirt and demersal organisms were the highest level in PFOA and PFOS.

1179 PFAS Tissue Distribution in Cattle

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Deciding when PFAS in beef cattle has declined sufficiently after exposure is stopped, such that the tolerable daily intake by high end meat consumers is not violated, requires information on clearance of PFAs from tissues (experimental) and a model for dietary intakes for cattle or from serum to tissue partitioning data. Belted Galloway beef cattle that had accumulated PFASs from water had blood sampled (tail vein) prior to stunning and exsanguination (as per standard abattoir practice) by a veterinarian. Animals were hung from water had blood sampled (tail vein) prior to stunning and exsanguination. Although there were the different patterns of the highest PFCs in each category, PFOA and PFOS were mainly detected in all categories. The dried seafood (including seasoned laver) and salt food were detected a high level of PFOA and PFOS compared with other categories. Also, the present study show that the distribution of the PFCs’ concentration were different as per biological classification, the raw material of samples, and habitat of the raw fish samples using statistical analysis. The result was that crustaceae, sea squirt and demersal organisms were the highest level in PFOA and PFOS.
The Interaction with and Transport of Three Perfluoroalkyl Sulfonates by Renal Organic Anion Transporters

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Perfluoroalkyl substances (PFAS) are environmentally resilient compounds that have been used in many industrial applications. Some PFAS have long serum elimination half-lives in humans with the transport activity of multi-drug transporters having a significant role in the retention and reabsorption of these compounds. We have demonstrated in the past that several of the renal organic anion transporters are involved in the disposition of perfluoroalkyl carboxylates. However, little is known regarding perfluoroalkyl sulfonates. Therefore, the aim of this study is to determine whether the human kidney organic anion transporters (OAT1, OAT3, OAT4, URAT1, and OATP1A2) influence the disposition of these compounds. We undertook this study by expressing different transporters transiently in HEK293 cells and measured the uptake of radiolabeled model substrates in the absence or presence of either perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), or perfluorooctane sulfonate (PFOS). The uptake of these compounds was also directly quantitated by LC-MS/MS. We observed that both PFBS and PFHxS inhibited transport activity of these selected transporters to a greater extent than PFOS, with the greatest inhibition observed in the transport activity of both OAT3 and OAT4. We determined that PFBS and PFHxS are substrates of OAT3 and OAT4 while PFOS is not a substrate of these transporters. This observation is consistent with the role of the kidney in handling and eliminating small (<450 MW) organic compounds. Within the limits of our study design, these results demonstrated that, in addition to perfluoroalkyl carboxylates, renal organic anion transporters preferentially interact and transport certain perfluoroalkyl sulfonates, suggesting a possible role in the retention and distribution of these compounds.

Accessing Toxicity Data for Per- and Polyfluoroalkyl Substances Using the US EPA CompTox Chemicals Dashboard


EPA’s National Center for Computational Toxicology is developing automated workflows for curating large databases within the DSSTox project, and providing access to chemical structure and toxicity data. The CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard), a publicly accessible web site providing access to data for ~760,000 chemical substances, the majority of these represented as chemical structures. The web application delivers a wide array of computed and measured physicochemical properties, in vitro high-throughput screening data and in vivo toxicity data, as well as integrated chemical linkages to a growing list of literature, toxicology, and analytical chemistry websites. In addition, several specific search types are in development to directly support the mass spectrometry non-targeted screening community, enabling cohesive workflows to support data generation for the detection and assessment of environmental exposures to chemicals contained within DSSTox. The application provides access to segregated lists of chemicals that are of specific interest to relevant stakeholders, including, for example, scientists interested in Per- and Polyfluoroalkyl Substances (PFAS). Added lists include those sourced from the European Union as well as developed in-house and now containing thousands of chemicals. A procured testing library of hundreds of PFAS chemicals, with a portion of the list annotated into chemical categories, has been integrated into the dashboard with a number of resulting benefits: a searchable database of chemical properties, with hazard and exposure predictions, and links to the open literature. This presentation will provide an overview of the dashboard, the developing library of PFAS chemicals and associated categorization, and efforts underway to develop new physicochemical property and environmental fate and transport Qsar prediction models developed for these chemicals. This abstract does not necessarily represent the views or policies of the US Environmental Protection Agency.

Development of a Maximum Allowable Dose Level for Perfluorooctane Sulfonate and Assessment of Exposures from Drinking Water in California

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Perfluorooctane sulfonate (PFOS), a polyfluoroalkyl substance with uses in stain-resistant coatings and fire-fighting foam, is known to be a prevalent contaminant in the environment as it is resistant to degradation. The US Environmental Protection Agency (US EPA) concluded that PFOS may be developmentally toxic to humans based on toxicology studies. Given these findings, PFOS was recently added to the California (CA) Proposition 65 list via the scientific mechanism. As there is now a Safe Harbor Level (SHL or Maximum Allowable Dose Level, MADL) for PFOS, the current work was undertaken to (i) derive a MADL and to (ii) determine if PFOS in drinking water is likely to exceed the MADL. CA Proposition 65 requires businesses to provide a clear and reasonable warning if they sell products that would result in chemical exposures above a SHL and it also prohibits a business from discharging effluent into drinking water sources if consumption of the drinking water would exceed the SHL. From the most sensitive and relevant PFOS reproductive study, a No-Observed-Effect Level (NOEL) of 0.1 mg/kg/d was identified with the critical effect being decreased weights in offspring. A MADL of 5.8 micrograms/d was calculated from the NOEL (0.1 mg/kg/d/1000 x 58 kg x 1000 µg/mg). Data from the Unregulated Contaminant Monitoring Rule were used to determine potential exposures to PFOS from drinking water in CA. PFOS was reported at an average and maximum concentration of 0.057 and 0.156 µg/l in CA, respectively, and a maximum concentration of 1.8 µg/l in all US. The higher concentrations are associated with industrial sites that manufacture or use polyfluoroalkyl substances, and are significant predictors of historical PFOS uses. Using exposure defaults from the CA Proposition 65 regulations (2 L/d), these concentrations would correspond to daily exposures of 0.114, 0.312 and 3.6 µg/d, respectively, which are all below the MADL. Thus, while the maximum concentrations would exceed the current US EPA lifetime drinking water health advisory of 0.07 µg/L (which was very conservatively derived and included a 20% relative source contribution adjustment), the MADL for PFOS for reproductive effects would not be exceeded. Based on the present work, the drinking water in CA from any source, which is reflective of historical uses of PFOS, would not be expected to exceed the calculated MADL for developmental toxicity.

Exposure of Nrf2-Deficient Astrocytes to PFOS and PFOA Results in Increases in ROS and Apoptosis

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Perfluoroalkyl substances (PFAS) include perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are environmentally persistent compounds used in many industrial and consumer products, such as fire-fighting foams, paints, and adhesives. These chemicals are found in measurable concentrations in human, animal and environmental samples. PFOS and PFOA have been detected in contaminated drinking water which could potentially cause long-term effects in humans and animals. Toxicity of these compounds, including hepatotoxicity, nephrotoxicity, and neurotoxicity has been demonstrated in several studies in various experimental models. The aim of this study was to determine the role of Nrf2 in protection against the neurotoxicity of these agents. Oxidative stress has been implicated in the activation of Nrf2, a transcription factor responsible for induction of antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase and reductase, and catalase. The in vitro models used in the study were Nrf2-/− and wild type C57BL/6 mouse astrocytes. Previous work in our laboratory has determined cytotoxicity using the Lactate dehydrogenase (LDH) Assay and morphological changes of exposed astrocytes by phase contrast light microscopy. Cytotoxicity was significantly greater in Nrf2−/− treated astrocytes as compared to wild type treated astrocytes via these methods. Current studies have now determined that the apoptosis was significantly induced in Nrf2−/− astrocytes as compared to wild type astrocytes after exposure to high doses (600µM and 800µM) of PFOS and PFOA, respectively, via caspase-3 assay. Additionally, the Nrf2−/− treated group exhibited higher ROS than the wild type group at the highest concentrations of PFOS and PFOA as determined by DCFDA fluorescent technique. These results indicate that Nrf2−/− astrocyte exposure to PFOS and PFOA results in significantly greater toxicity than matched wild type astrocytes suggesting a role for oxidative stress in the observed neurotoxicity.
Perfluorobutane sulfonate, PFBS, is a perfluorooctylated substance and a shortened (4-carbon) alternative of PFOS, perfluorooctane sulfonate (8-carbon). PFBS has a shorter half-life and lower reported toxicity than PFOS. Exposures in zebrafish embryos have shown it to affect pancreatic islet morphology and other developmental endpoints, but the critical exposure window prior to conception has not yet been examined. Using the zebrafish model (Danio rerio), this pilot study aims to investigate the effects of a maternal, prenatal exposure to PFBS during early developmental endpoints. Three adult female fish, Tgβ-gal/Fgf, were stripped to remove existing eggs and exposed to 0.25 µg/mL PFBS or 0.01% DMSO for 1 week during a cycle of oocyte maturation. Following exposures, female fish were bred daily with unexposed male fish for a period of two weeks. Embryos were imaged daily through 20 days post fertilization, hpf, via live brightfield and fluorescence microscopy, and total larval length, yolk sac area, and pancreatic beta cell area were measured. Additionally, pooled samples of 25 embryos (3 hpf) or larvae (120 hpf) were assessed for total protein, triglycerides, cholesterol, and glucose using commercially available kits. In newly fertilized eggs (3 hpf), there was a significant 7% reduction in yolk sac area in the embryos collected from the 0.25 µg/mL PFBS-exposed maternal group compared to controls. These embryos also had a significant 50% decrease in cholesterol. At 24 hpf, embryos from PFBS-exposed females had significantly decreased yolk sac area (8%), which persisted at 5 dpf (11% reduction). In addition, at 5 dpf, the 0.25 µg/mL PFBS larvae had a significant 13% increase in pancreatic beta cell area that corresponded with a trend towards lower larval glucose content, as would be expected with increased islet size and capacity. This study demonstrates that embryos are developmentally impacted by maternal exposures to PFBS during oocyte maturation. The exposure in female fish may impact the nutritional deficit that is known to occur during development, which is evident in the effects on cholesterol and yolk sac area in the early developmental stages. This nutritional deficit, which is possibly coupled with loading of toxicant into the eggs, may explain the persistence of effects at 5 dpf. Together this suggests that there may be alterations in oocyte maturation and metabolic homeostasis in larvae associated with maternal, preconception exposure to PFBS. This work was supported by R01ES028201.

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### 1184 Maternal Preconception Exposure to PFBS Alters Nutrition and Growth of Offspring

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Legacy per- and polyfluoroalkyl substances (PFAS) have been shown to affect development and puberty in mice. This pilot study was conducted to compare the effect of a replacement PFAS, GenX (ammonium perfluoro-2-methyl-3-oxahexanoate), with effects of a legacy compound, perfluorooctanoic acid (PFOA) on developmental end points and mammary gland morphology in female mice offspring. Time-pregnant CD-1 mice were dosed with either 1 or 5 mg/kg PFOA, or 2 or 10 mg/kg GenX (blindly allocated) from gestation days 1.5 to 17.5. Litter weights were recorded at PND 0.5, and at PND 2.5, when litter size was equalized to 10 pups. Individual pup weights were recorded on PND 6, 9, 12, 21, and 36. The PND of fur appearance, eye opening, and incisor eruption were recorded. Pups were necropsied at either PND 21 or 36 for tissues and serum. Dams were euthanized at PND 21 and uteri were analyzed for implantation sites for calculation of resorptions. Compared to the control group, body weights were significantly decreased in the 5 mg/kg PFOA group at PND 0.5, 2.5, 6, and 9. 1 mg/kg PFOA reduced pup body weight at PND 2.5, and 10 mg/kg GenX reduced pup body weight at PND 6, with no further weight changes noted. Relative liver weights were similar to control at PND 21 and 36. Eye opening was delayed in the 5 mg/kg PFOA group. Puberty delays were evident in female pups in both PFOA and 10 mg/kg GenX groups. Mammary gland development was stunted in all dose groups of GenX and PFOA. Mammary glands were scored blinded to treatment on a scale of 1 to 4 by two reviewers for stage of development, with 4 being the most fully developed glands. At PND 21 control glands were scored an average of 3.5. 2 mg/kg and 10 mg/kg GenX and 1 mg/kg PFOA averaged scores between 1.5 and 2 and displayed limited branching, lack of ductal growth, and fewer terminal end buds. Weaning 5 mg/kg PFOA glands were scored as a 1 and 1.5 had very limited ductal growth. At PND 36, controls averaged a 3.5 score and all PFAS dose groups averaged a 2.5. Even though GenX is thought to exhibit rapid elimination in mice and PFOA persists for weeks, the doses tested here suggest that GenX is more toxic than PFOA on a mg/kg exposure basis when assessed during development.

### 1185 PFAS Clearance in Cattle

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Farms are often located near sources where PFAS firefighting foams were used (e.g., training grounds of rural fire services, defence bases, buffer areas of oil/gas refineries). However, there is limited information available that facilitates management of PFAS affected farms or the animals. The objectives were to gather robust information on serum clearance of PFAS from beef cattle, tissue distribution, partial partition coefficients, and GIT absorption. A herd of Belted Galloway beef cattle (16 heifers and 3 steers) were exposed to PFAS in water for 2 years. - All had access to the same water, constant PFAS concentration, no other water source. 1. All animals bred on property, or larvae (120 hpf) were assessed for total protein, triglycerides, cholesterol, and glucose using commercially available kits. In newly fertilized eggs (3 hpf), there was a significant 7% reduction in yolk sac area in the embryos collected from the 0.25 µg/mL PFBS-exposed maternal group compared to controls. These embryos also had a significant 50% decrease in cholesterol. At 24 hpf, embryos from PFBS-exposed females had significantly decreased yolk sac area (8%), which persisted at 5 dpf (11% reduction). In addition, at 5 dpf, the 0.25 µg/mL PFBS larvae had a significant 13% increase in pancreatic beta cell area that corresponded with a trend towards lower larval glucose content, as would be expected with increased islet size and capacity. This study demonstrates that embryos are developmentally impacted by maternal exposures to PFBS during oocyte maturation. The exposure in female fish may impact the nutritional deficit that is known to occur during development, which is evident in the effects on cholesterol and yolk sac area in the early developmental stages. This nutritional deficit, which is possibly coupled with loading of toxicant into the eggs, may explain the persistence of effects at 5 dpf. Together this suggests that there may be alterations in oocyte maturation and metabolic homeostasis in larvae associated with maternal, preconception exposure to PFBS. This work was supported by R01ES028201.

### Do Perfluoroalkyl Carboxylic Acids Interact with the Bile Acid Transporter NTCP?

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Sodium/taurocholate cotransporting polypeptide (NTCP) is a sodium-dependent bile acid transporter located at the basolateral membrane of hepatocytes. NTCP plays an important role in the enterohepatic circulation of bile acids and this mechanism has been suggested to be a possible explanation as to why certain perfluoroalkyl substances have long serum elimination half-lives in humans. We had previously demonstrated that some perfluoroalkyl sulfonates can be transported by NTCP, however, little is known about perfluoroalkyl carboxytes. Therefore, the purpose of this study was to determine if perfluoroalkyl carboxylic acids would interact with NTCP and potentially act as NTCP substrates. Human embryonic kidney cells (HEK293) stably expressing NTCP were plated on poly-D-lysine-coated 24 well plates, and uptake of sodium-dependent 1H-taurocholate was measured between 24 and 48 hours after plating in the absence or presence of perfluoroalkyl carboxylic acids with varying chain lengths from 3 to 18 carbons at either 10 or 100μM. Direct uptake was also quantitated by LC-MS/MS. Protein concentrations were determined by BCA assay. Perfluorooctanoic acid (PFOA), perfluoromonoanoic acid (PFNA), and perfluorodecanoic acid (PFDA) are the strongest inhibitors of NTCP-mediated taurocholate uptake with IC50 values of 8.7, 12.0 and 9.2μM, respectively. All three compounds are also transported by NTCP. Interestingly, decanoic acid, the C10 medium-chain fatty acid analogue of PFDA, inhibited NTCP-mediated transport only by about 30% and is not a substrate of NTCP. Similar to perfluoroalkyl sulfonates, certain perfluoroalkyl carboxylic acids can interact and inhibit NTCP-mediated taurocholate uptakes. The strongest inhibitors are ones with longer than 12 carbons and act as substrates of NTCP. Under the study conditions, our data provided evidence that longer chain perfluoroalkyl carboxylic acids such as PFOA, PFNA, and PFDA can undergo enterohepatic circulation.
Perfluoroalky acid (PFHxA) is a short-chain, six-carbon perfluoroalky acid (PFAA) and is a primary impurity, degradant, and metabolite associated with the short-chain fluorotelomer-based chemistry used in the United States and Europe today. The transition to short-chain fluorotelomer-based products as a cornerstone in replacement fluorochemistry raises questions regarding potential human health risks associated with exposure to fluorotelomer and PFHxS. Here, we present a critical review of data relevant to such a risk assessment, including epidemiological studies and in vitro and in vivo toxicity studies that examined acute, subchronic, and chronic toxicity of PFHxA. Key findings from toxicokinetic and mode of action studies are also evaluated. Sufficient data exist to conclude that PFHxA is not carcinogenic, is not a selective reproductive or developmental toxicant, and does not disrupt endocrine activity. A chronic human-health-based oral reference dose (RfD) for PFHxA of 0.2 mg/kg-day was calculated using benchmark dose modeling of renal papillary necrosis from a chronic rat bioassay. This RfD is four orders of magnitude greater than the chronic oral RfD calculated by the US Environmental Protection Agency for perfluorooctanoic acid (PFOA). The PFHxA RfD can be used to inform public health decisions related to PFHxA and fluorotelomer precursors for which PFHxA is a terminal degradant. Examples provided herein include calculations of protective human health-based screening levels, including a lifetime health advisory and threshold limit value, that provide protective screening level protective of sensitive subpopulations. Occurrence studies demonstrate that the range of environmental concentrations of PFHxA at sites with identified sources of PFAA contamination are at least an order of magnitude lower than screening levels. Furthermore, human serum and urine biomonitoring studies demonstrate that low serum PFHxA levels and rapid elimination kinetics of PFHxA and fluorotelomer precursors contribute to a high margin of safety for the general population. The analyses herein can support site-specific risk assessments as well as product stewardship initiatives for current and future fluorotelomer-based chemistries.

Perfluorooctane sulfonate (PFOS), a commonly used ingredient in aqueous film forming foams, fabric protectors, stain repellents, and other surface-coating products, has the highest environmental and biological persistence among per- and polyfluoroalkyl substances (PFAS). In fact, PFOS is ubiquitously detected in wildlife and the general human population across the world due to its biopersistence and bioaccumulation. Furthermore, given the growing scientific concern regarding its potential toxicity, there have been international regulations, advisories, and guidelines to limit exposure that are constantly evolving based on current scientific knowledge. Thus, in this presentation, we critically evaluate international and domestic regulatory values, and their corresponding methodologies, in order to gain insight into the dose response assessment approaches that have been adopted for PFOS to date. Here, we found that the international and domestic regulatory values, there are large differences in the reference doses (RfD) determined, ranging from 0.0018 to 0.3 µg/kg bodyweight (BW)/day. This was due to a range of aspects, including differences in study selection and thus species, as well as pharmacokinetic considerations and applied uncertainty factor(s). In particular, a total of 23 studies from the international and domestic regulatory agencies that selected the same study, some still had distinct RfD values due to differences in the application of uncertainty factors and pharmacokinetic considerations. Furthermore, it was found that the lowest regulatory value based on a study in mice generated the most conservative RfD, whereas in general regulatory values based on studies in rats were less conservative and those based on studies in monkeys were the least conservative. Overall, while the range of RfDs identified for determination of the regulatory values was considerable, there was a much narrower range for the point of departure in animal studies, indicating that the treatment of values after study selection is the most significant factor accounting for the large range of RfDs. This critical analysis may be used to identify the best practice for the evaluation of dose response for PFAS.

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Perfluoroalkanesulfonate (PFHxSs) and perfluorooctanoate (PFOA) are perfluoroalkyl compounds that are persistent and bioaccumulative. Some toxicological studies had reported reductions in the measurements of serum free thyroxine (FT4) in rodents with exposure to either PFOA or PFHxS; however, these studies did not take into account the possible binding competition that would result in a negative bias associated with the analog FT4 immunoassays used. Several PPARα agonists, including perfluorooctanesulfonate (PFOS), have been shown to compete and displace serum thyroxine (T4) from binding proteins. Given their structural similarity to PFOS, it is plausible that PFHxS and PFOA can also compete and displace T4. We tested this hypothesis in vitro by incubating mouse, rat, and human sera with 0 - 500 µM of PFHxS or PFOA. Serum FT4 was directly quantitated with LC-MS after equilibrium dialysis. Serum FT4 levels positively correlated with increasing PFHxS concentrations in mouse and rat sera. At 500 µM, they were approximately 200% and 650% over controls, respectively. FT4 levels also positively correlated with increasing PFOA concentrations in mouse and rat sera (~ 250% and 1000% over controls, respectively, at 500 µM). While there was no significant concentration-dependent increase in FT4 in human sera with PFHxS, there was a slight FT4 increase with PFOA. The degree of T4 displacement by PFOA in human sera (~ 30% over control at 500 µM) was much lower in magnitude than rodents. Compared to PFHxS, the higher displacement of T4 by PFOA appeared to be serum specific; whereas the higher displacement with PFHxS was species independent.

Using high PFHxS or PFOA concentrations that are several orders of magnitude higher than the general population, our data demonstrated that (1) PFHxS and PFOA can displace serum FT4 from binding proteins in vitro; and (2) rodents are more prone to serum T4 displacement than humans.

Integrating genomic data from short-term exposure to identify the mode of action (MoA) and point of departure (POD) for a compound’s quantitative risk assessment requires the evaluation of both dose-response and time-course results for the MoA key events. We performed a hepatic transcriptomic analysis on rats treated with PFOA to unravel the potential molecular mechanisms underlying the observed key events. We also investigated the dose-response and time-course concordance of transcriptional and apical liver effects. Male SD rats were treated with 0, 1, 5 or 15mg/kg PFOA by oral gavage for 7, 14, and 28 days. Hepatic total RNA were isolated for RNA sequencing experiments. Ingenuity and KEGG pathway analyses suggested that PFOA modulated the PPARα signaling (the first key event) in a dose-dependent manner at all time points studied. Using a William trend test, a total of 779, 829, and 743 genes showed dose-response pattern after 7, 14, and 28 days of PFOA treatment, respectively. Only 16 genes differentially expressed in the low dose PFOA treatment after 7d compared to control. The repression of Cyp4a11, Aco1, Aco2, Vnn1, and Nr1d1 showed a dose-response and time-dependent upregulation trend, suggesting these transcripts as potential biomarkers for screening for PPARα mediated compounds. The median benchmark dose estimates (BMDL50) of Aco1 were concordant with apical benchmark doses (BMDL50) for acyl-CoA oxidase activity at all time points studied. However, the median BMDL50 in the whole “peroxisome lipid metabolism” pathway appeared to underestimate the potency of PFOA on the PPARα activation. No dose-dependent change was found on the proto-oncogene expression including Myc, Mki-67, and Pcn1. Interestingly, Reactome pathway analyses identified a subset of genes with dose-responsive behavior that related to the S phase in the cell cycle. These data correlate with our previously observed increased hepatocytes DNA synthesis at 7d (BrDU) but lack of cell division (Ki67). The median BMDL50 of these genes was 2-fold higher than the BMDL50 for BrDU labeling index. Taken together, results from both apical endpoints and transcriptomic analyses demonstrated that PFOA induced a clear dose- and time-dependent activation of PPARα. However, PFOA only induced a transient increase in DNA synthesis in rat liver without any sign of driving cell proliferation, suggesting that further investigation is needed for the second key event in the MoA of PFOA induced rat liver tumor formation.
Perfluorooctanoic acid (PFOA) is a synthetic, fluorinated organic acid previously used in the production of fluoropolymers in the United States, and is a byproduct found in a variety of consumer products that contain fluoropolymers or have fluoropolymer coatings. PFOA has been a recent focal point for regulation due to the widespread presence of the chemical in drinking water wells throughout the United States. Water resources impacted by PFOA have been associated with releases from manufacturing sites, municipal wastewaters, and industrial or municipal landfills where treated products have been disposed. In 2016, the US EPA issued a lifetime drinking water Health Advisories (HA) for PFOA of 0.07 micrometers per liter (µg/L). The goals of the current study were to: 1) determine the basis for the health-based guidelines (state and federal) for PFOA in drinking water, and 2) evaluate the assumptions used by the US EPA in setting the drinking water advisory limit. A total of nine PFOA and biofluids. We reviewed the acute, sub-chronic and chronic toxicity of PFOA in mammalian, avian, reptilian and amphibian species to derive toxicity reference values (TRVs) that could be protective for species in those classes. Despite limited wildlife exposure data, available laboratory animal studies enabled identification of target organs, dose-response endpoints, and NOAEL and LOAEL values. The US Army Public Health Center Technical Guide 254 - Standard Practice for Wildlife Toxicity Reference Values, guided our development of class-specific TRVs. Although considerable mammalian toxicity data were available, comparatively few data were available for avian, amphibian species, and severely limited data was available for reptiles - limiting meaningful TRV derivation for these classes. Tentatively derived oral TRVs were based on the most sensitive mammalian species. For murine immunological effects, a LOAEL of 3.75 mg/kg/day and a NOAEL of 1.88 mg/kg-day were derived. Benchmark dose (BMD) analysis of antigen-specific IgM responses, indicative of suppressed immunity, gave a BMD of 3.06 mg/kg-day. Further, one standard deviation (1SD) from the control mean was selected as the benchmark response (BMD(1)) level for continuous data to give a lower bound 95 percent confidence limit (BMDL) of 1.75 mg/kg-day. Selected inhalation TRVs for the class Mammalia gave a NOAEL of 0.1 mg/m³ and a LOAEL of 1 mg/m³ with moderate confidence from sub-chronic studies conducted in rats. By comparison, selected dermal TRVs for the class Mammalia gave a NOAEL of 0.075 mg/kg and a LOAEL of 0.125 mg/kg with very low confidence. The available toxicity data on avian, amphibian and reptilian species were insufficient to develop TRVs. These initial TRVs were based on the most sensitive species available, which might be formally protective of the entire class of mammalian wildlife species in ecological risk assessment.

Bioaccumulation patterns for persistent organic pollutants (POPs) in humans are influenced in part by diet, lifestyle and socio-economic factors. Moreover, persistence and bioaccumulation in hepatic metabolic pathways. Including ingestion of POPs in lipid metabolism, oxidative stress, inflammation, TCA cycle, glucose and amino acid metabolism. Changes in the metabolome correlated with changes in genes that regulate these pathways. Integrative analyses also demonstrated a strong correlation between the alterations in microbiota composition and hepatic metabolic pathways. The main findings from the study demonstrated that the molecular and biochemical changes induced by PFOS are mediated in part by the gut microbiome, which alters gene expression and the host metabolome in mice.

Perfluorooctane sulfonate (PFOS) is a persistent environmental chemical whose biological effects are mediated by multiple mechanisms. Recent evidence suggests that the gut microbiome may alter the fate and effects of environmental chemicals in the host. Thus, the aim of this study was to determine whether PFOS influences the gut microbiome and host metabolism. Male C57BL/6 mice were fed a control diet with and without 0.003%, 0.006% or 0.012% PFOS. 16S rRNA gene sequencing, metabolomic, and molecular analyses were used to examine the gut microbiota of mice after dietary PFOS exposure. Dietary PFOS exposure caused a marked change in the gut microbiome compared to controls. Dietary PFOS also caused dose-dependent changes in hepatic metabolic pathways. Including integration of gut microbial expression and exposure, and the results indicates that ACHS females had higher POPs body burden. The occurrence of liver disease in participants of the Anniston Community Health Survey (ACHS), a historic cohort with exposures to multiple POPs, primarily polychlorinated biphenyls (PCBs). The current study aims to investigate if sex differences exist with serum POPs levels in the ACHS baseline (I) and follow-up (II) samples. The study also investigated if associations between POPs and liver disease biomarkers in the ACHS population are sex-dependent. Structural and functional groupings of PCBs congeners were tested for differences across sex with adjustment for lipids only or adjusted for age, BMI, total lipids, race/ethnicity, diabetes, drinking and smoking status for ACHS I (517 females, 221 males) and II (245 females, 93 males). Sex differences for levels of organochlorine pesticide (OCPs) and liver disease biomarkers were also assessed in ACHS I. Generally, females had higher levels of PCBs compared to males. Total mono-ortho PCB levels were higher for females in both ACHS I and II. Further, the non-ortho, dioxin-like PCBs (e.g. 81, 126, 169, 189) were higher in females at follow-up. Additionally, females in ACHS II had higher estrogen-like (types 1 and 2), anti-estrogenic and thyroid-like PCB levels. In terms of disease biomarkers, females from ACHS I exhibited higher leptin, adiponectin and total cholesterol vs. males. Importantly, these females had higher levels of OCPs including hexachlorobenzene, oxychlordane and DDT. Generally, in terms of PCB load, females had higher concentrations of PCB congeners with lower chlorine substitutes while males had higher levels of congeners with higher number of chlorine substitutes. Taken together, the results indicated that ACHS females had higher POPs body burden. The findings warrant further investigations into sex-based disease outcomes with pollutant exposures.
Persistent organic pollutants (POPs) including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,7,8-tetrachlorodibenzofuran (TCDF), and polychlorinated biphenyls have been extensively studied. However, how the intestinal microbiome and the systemic effects of POPs in rodent models to better clarify the host-microbiome relationship in the toxicity response, we explored connections between changes in the microbial metatranscriptome and metabolome with host toxicity. Metatranscriptomic analysis of the mouse intestinal cecal microbiome after a 5-day exposure to low dose TCDF (5 µg/kg) or high dose TCDF (24 µg/kg) was conducted. SAMSA2 (Simple Annotation by Metatranscriptomes by Sequence Analysis) identified enzymes and pathways directly enriched or depleted after treatment of TCDF including increased amino acid metabolism (LDA score = 2.94, P Value < 0.05) increased serine-glycine-oxytate cycle (LDA score = 3.03, P Value < 0.05) and decreased glycogen metabolism (LDA score = 3.06, P Value < 0.05) after TCDF treatment in the microbiome. Additionally, 16S rRNA and metagenomics sequence analysis were integrated with 1H NMR and high resolution Orbitrap mass spectrometry metabolomics data to predict the changes that were seen with the metatranscriptomic analysis. This investigation not only elucidated the systemic metabolic changes that were directly caused by TCDF modulation of the intestinal microbiome but also provided validation for the predictive techniques used to understand the toxic interaction with the microbiome. Importantly, this study provides convincing evidence that establishing microbiome toxicity endpoints may help to better inform risk assessment.

Physiologic and Metabolic Impact of Persistent Organic Pollutants on Gut Microbiota

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Emerging evidence supports that exposure to persistent organic pollutants (POPs) can impact gut microbiota-host metabolic homeostasis, leading to metabolic disorder. Here, we examined the direct effects of POPs including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,7,8-tetrachlorodibenzofuran (TCDF), and polychlorinated biphenyl (PCB)-123 on the gut microbiota using in vitro models. Mouse cecal microbiota were incubated with three doses of TCDD (high: 0.2 µg/ml, middle: 0.02 µg/ml, and low: 0.002 µg/ml), TCDF (high: 2 µg/ml, middle: 0.2 µg/ml, and low: 0.02 µg/ml), and PCB (high: 2 µg/ml, middle: 0.2 µg/ml, and low: 0.02 µg/ml) for 4 h in an anaerobic chamber. NMR- and mass spectrometry-based metabolomics combined with flow cytometry was used to evaluate the direct physiologic and metabolic impact of TCDD, TCDF, and PCB on the microbiota. TCDD, TCDF, and PCB treatment all resulted in significant decreases in microbial metabolic activity following POPs treatment in a dose-dependent manner (decrease from 62.7 ± 6.4% [vehicle] to 53.6 ± 1.6% [TCDD], 55.0 ± 0.6% [TCDF], and 52.9 ± 1.1% [PCB]). Global and targeted 1H NMR analyses revealed significant changes in microbial metabolites including dose-dependent increases in microbial lipids (increase 1.18-fold [TCDD], 1.16-fold [TCDF], and 1.19-fold [PCB]) and decreased amino acids, lactate, and lactate after TCDD, TCDF, and PCB treatment. These data provide new insights into the direct role of POPs on the gut microbiota and begin to establish possible microbial toxicity endpoints which may help to better inform risk assessment.

Chronic Exposure to Tetrabromobisphenol A Potentially Alters Circadian Rhythm in Rats

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Tetrabromobisphenol A (TBBPA) is a brominated flame retardant used as a component in the epoxy resin of circuit boards for electronic devices. It has been found at higher levels inhous dust, and thought initially to be not present in health risks, more recent studies have indicated a potential link between higher exposure levels and uterine tumors in rats, as well as suggesting that it may have endocrine disrupting function. The International Agency for Research on Cancer (IARC) determined in 2016 that there was sufficient evidence to list TBBPA as probably carcinogenic to humans (Group 2A). Previous studies in our lab using Wistar Han rats treated with 250 mg/kg/d for 5 days linked TBBPA exposure to disruption in estrogen homeostasis and potentially thyroid and immune system function. RNA-Seq analysis of liver and uterus from the TBBPA and vehicle-treated rats also indicated a possible effect on expression of the genes controlling circadian rhythm. To investigate this, expression levels of the core circadian genes Clock, Arntl (Bmal1), Npas2, Period (Per) 1, 2, and 3, and Cryptochrome (Cry) 1 and 2 were measured in liver and uterus from these rats using droplet digital PCR (ddPCR). After accounting for differences in time of euthanasia, results showed a possible shift of the gene expression in the uterus but not the liver of the animals treated with TBBPA. For the genes that were expected to alter the estrus cycle and are representative of possible pathways for perturbation of estrogen levels in the uterus, as well as affecting expression of numerous other genes which are reliant on circadian signals to regulate expression. This research was supported by the Intramural Research Program of NIH/NIC (Project ZIA BC 011476).

Mitigating the Health Consequences of Paternal Exposure to Arctic Pollutants with Folic Acid

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Traditional diets of Inuit people result in low folate intake and high body burdens of Persistent Organic Pollutants (POPs), known to have negative health effects. Indeed, Inuit have more adverse pregnancy outcomes and shorter life expectancies than non-Inuit Canadians. Recent studies have shown that the father’s lifestyle influences his offspring health. Therefore, we hypothesized that Folic acid (FA) supplementation might protect paternal health in the presence of POPs. In this study, we evaluated the direct physiologic and metabolic impacts of POPs at the intestinal level of the father and male offspring and explored the potential relationship between the paternal POP burden, FA supplementation, and fetal development. The study provided evidence that FA supplementation may protect against POP-associated health effects in the offspring of fathers with high POP burdens.

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. TL has been associated with higher risks for several types of cancer. The National Toxicology Program (NTP) conducted two year studies exposing female Sprague Dawley rats to various doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the dioxin-like compounds (DLC) PCB 126 and PCB 118, the non-DLC PCB 153, and a mixture of PCB 126 and PCB 153. 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 banked liver and lung tissue obtained from the NTP studies and RTL was assessed by quantitative PCR. Relative to time-matched vehicle controls, liver RTL increased 3-10% and 15-24% in rats given various doses of TCDD for 13 weeks and 52 weeks, respectively. Similarly, RTL increased 7-13% and 17-27% in the liver and increased 6-15% and 14-33% in the lung following exposure to PCB 126 for 13 weeks and 52 weeks, respectively. Rats dosed with PCB 118 showed increases in liver RTL of 2-17% and 15-30% and increases in lung RTL of 8-20% and 15-18% following 13 week and 52 week exposures, respectively. Following 13 weeks of exposure to the mixture of PCB 126 and PCB 153, RTL increased 9-11% in the liver and 12-16% in the lung. After 52 weeks of exposure to the mixture of PCB 126 and PCB 153, liver RTL increased 24%, while lung RTL increased 6-28%. In contrast, RTL decreased from 4-9% in the liver of rats receiving PCB 153 alone. The association between RTL and test compound is congener-specific and associated with the varying toxicological activity of PCB congeners. An increase in RTL was observed in rats treated with the DLCs, TCDD, PCB 126, and PCB 118, and the mixture of PCB 126 (DLC) and PCB 153 (non-DLC). These rats also showed an increased incidence of cancer and other non-neoplastic lesions in the liver, lung, and other organs. In contrast, a general reduction in RTL was observed in rats treated with PCB 153 alone, which exhibited hepatic hypertrophy and other non-neoplastic lesions. Increases in RTL may be an early indicator of carcinogenesis that occurs following two years of exposure to DLCs. Supported in part by the intramural research program of NCI/NIH.

1201 Transcritomic Analyses of Ortho-PCB Exposures in Larval Zebrafish


Developmental effects of PCBs are among the most important and least well-understood concerns in PCB toxicology. Ortho-substituted PCBs are far more abundant than the dioxin-like planar non-ortho-PCBs, and consequently are still found in significant concentrations in human maternal samples. In this study, we used zebrafish larvae to investigate the transcritomic effects of neurotoxic PCBs developing vertebrata. PCB52, PCB95, and PCB153 were not acutely toxic to zebrafish in nominal water-borne concentrations up to 1 μM. However, ortho-substituted PCB153 and PCB95 alter the behavior of 6 days post-fertilization (dpf) zebrafish larvae in standard light-dark movement assays. Both PCBs caused an increase in light-stimulated freezing, and a decrease in dark induced activity following exposures from 4 hours post-fertilization (hpf). To examine more acute effects of ortho-PCB exposure, transcriptomic analyses of zebrafish larvae exposed from 48-72 hpf were performed. Similar numbers of genes were differentially regulated by PCB153 and PCB95 after 24 hrs. PCB153 dysregulated 265 genes (90 up- and 175 down-regulated), while PCB95 altered 89 genes. PCB153 down-regulated CYP activity, glycosylation/glucogenesynthesis, and lipid binding and transport, and CYP2A4s were prominently down-regulated. PCB95 strongly dysregulated expression of apolipoprotein (apoA and M) and up-regulated genes for fatty acid and cholesterol binding and transport. In contrast, these short exposures to moderate concentrations of PCB95 significantly altered only 5 genes, with only zona pellucida glycoprotein expression being significantly up-regulated. The low number of genes dysregulated by PCB95 and 52 in this study may be a result of significant variation in the control, DMSO-exposed fish. Ongoing work on targeted transcritomic analyses of larvae will provide important information on the mechanisms of these environmental toxicants. NIH SPA2ES007381. The Boston University Superfund Research Program.

1202 Skeletal Toxicity of Co-planar Polychlorinated Biphenyl Congener 126 in the Rat Is Aryl Hydrocarbon Receptor-Dependent

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Polychlorinated Biphenyls (PCBs) are persistent organic pollutants which bioaccumulate up the food chain. There is epidemiological evidence linking this to decreased bone mineral density, however mechanisms whereby PCBs produce bone loss are poorly studied. PCBs are complex mixtures of ortho- and co-planar congeners. The toxicity of co-planar PCBs have been suggested to be associated with activation of aryl hydrocarbon receptor (AhR). The hypothesis underlying this study is that co-planar PCB 126 acts on the skeleton via the AhR. PCB 126 (5 umol/kg) or corn oil control was administered to N=3-6 male and female, Wild Type (WT) and AhR-/- rats via intraperitoneal injection. Animals were sacrificed after four weeks, bone length was measured and bone morphology was assessed by micro-computed tomography.

WT rats exposed to PCB126 had reduced serum calcium, tibia length, cortical total area and medullary area relative to vehicle controls (P<0.05), with an increased effect in females relative to males. There were no significant effects on cortical thickness or trabecular parameters. Reduced bone length was the only genotype specific effect (P<0.05). In contrast, the effects of PCB126 on bone parameters was abolished in AhR-/-animals. The data suggests that effects on cortical bone loss may be mediated by AhR.

Studies will clarify importance mechanisms underlying skeletal toxicity of dioxin-like PCBs and highlight potential therapeutic targets to combat bone loss. Supported in part of PREP R25GM12189 and NIH-R37AA10282 (MJR).

1203 Toxicity of PCB126 in Adult Male and Female Rats, Including Timed Pregnant Rats

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PCB126 is the most potent dioxin-like toxicant among PCBs. It binds to the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor involved in the regulation of biological responses to planar aromatic hydrocarbons. The AhR regulates many xenobiotic-metabolizing enzymes, such as CYP1A1. PCB126 is not only hepatotoxic, but also a reproductive and developmental toxin. Our hypothesis is that PCB126 is more toxic in utebro than in adult male rats. To test this, we used AhR/- only (AhR KO) rat model created using CRISPR/Cas9, and a timed pregnant rat study. Comparisons were made between wild type (WT) and AhR KO male and female Holtzman Sprague Dawley rats and Sprague Dawley pregnant rats (dams). In AhR KO study, rats received a single IP dose of corn oil (5ml/kg) or PCB126 (.5umol/kg) in corn oil and necropsied the next day 12 and 24 h. In pregnant rat study, dams were injected with a single IP dose of corn oil (5ml/kg) or different doses of PCB126 (0.5, 1.0, 2.0, 5.0, 5μmol/kg) on gestation day 12 and necropsied after 6 days. Both, PCB126 exposed WT rats and dams had significant weight loss (P<0.05) compared to AhR KO rats. Similarly, relative thymus weights were lower (P<0.05) and relative liver weights were higher (P<0.05) in WT rats and dams compared to PCB126 exposed AhR KO rats. In addition, PCB126 exposed WT rats had decreased serum glucose levels, while there was no effect on serum glucose in the AhR KO rats and dams. Also, relative ovarian weight was decreased in female WT rats exposed to PCB126, but no significant change was seen in exposed AhR KO rats and dams. In contrast, serum estradiol level was unaffected by PCB126 in WT and AhR KO female rats, while PCB126 exposed dams had significantly increased serum estradiol. Moreover, the high dose of PCB126 (5μmol/kg) decreased average fetal number, and increased the implantation sites, suggesting fetal death and loss, but no adult rats died within the first 2 weeks after receiving the same dose. All effects in the dams were dose-dependent. These data suggest that most, if not all, toxic effects of PCB126 are mediated through the AhR and that even short time (6 days) in utebro exposure is extremely toxic, possibly due to a dysregulation of the estradiol homeostasis. Funded by NIHES P24ES053661; HD020676; HD079363.

1204 Beneficial Effect of Resveratrol in Combined Treatment with Cisplatin on Growth Inhibition and Apoptosis Induction in Gastric Cancer SGC-7901 Cells

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Cisplatin, also known as cis-dichlorodiammine platinum (DDP), is a commonly used chemotherapy drug in clinical treatment of gastric cancer; however, the drug has been shown to have a dose-dependent toxicity to body systems. To reduce its toxicity and enhance chemotherapeutic efficiency, it is desirable to co-administer non-toxic compound(s) to reduce the dosage of cisplatin while maintaining its anti-cancer effect. Resveratrol (RES) is a polyphenol compound isolated and extracted from grapes, blueberries and other Chinese herbal plants such as polygonum giant knotweed and mulberry. This study was designed to use cultured gastric cancer SGC-7901 cells to test the hypothesis that co-treatment of RES with cisplatin may enhance the latter’s efficacy in inhibiting the cancerous growth and in induction of apoptosis in SGC-7901 cells. Experiments were divided into 6 groups: control without drugs, RES-only (50 μM), RES combined with low and medium-cisplatin groups (50 μM RES+1 μg/mL and 4 μg/mL DDP, respectively), mid-dose cisplatin-only (4 μg/mL DDP), and high-dose cisplatin only (6 μg/mL DDP). Twenty-
four hours after various treatments in cultured cancer cells, the MTT assay showed that the growth inhibition in the low-dose DDP group treated with RES was significantly higher than that of the mid-dose DDP alone. Similarly, the RES treatment in the mid-dose DDP group achieved a more significant inhibition of cancer cell growth than the high-dose DDP alone. Data by the flow cytometry indicated that the apoptosis in the low-dose DDP treated with RES was significantly higher than that by the mid-dose DDP alone, and the effect of RES in the mid-dose DDP was higher than that of the high-dose DDP alone. Results of cell cycle detection further showed that RES in the low-dose DDP group blocked SGC-7901 cells in G0/G1 phase, which was similar to the effect of the treatment by the mid-dose DDP alone; the combination treatment with RES and the mid-dose DDP achieved the similar effectiveness by the high-dose DDP alone. Taken together, our results suggest that RES appears to show a beneficial anti-cancer therapeutic effect in combined treatments with cisplatin; the effect is due to RES’s enhancement of cisplatin’s inhibiting growth and inducing apoptosis in cancerous SGC-7901 cells.

1205 Metformin Scavenges Methylglyoxal to Form a Product That Improves Endothelial Cell Function: Potential Novel Mechanism of Metformin Action

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Reactive dicarbonyls, such as methylglyoxal (MG), are highly elevated in type-two diabetes mellitus (T2DM) patients. These endogenous electrophiles covalently react with proteins to form non-enzymatic advanced glycation end products (AGEs), which are major causes of cellular damage and dysfunction during diabetic cardiovascular complications. The T2DM first-line drug therapy, metformin (MF), significantly reduces adverse diabetic endpoints more effectively than other antihyperglycemic agents. The exact mechanism(s) by which metformin inhibits cardiac maladies is far from well characterized. We previously discovered that metformin scavenges MG to form a novel imidazoline (IMZ) metabolite, thus reducing MG-related AGEs. Many compounds that possess an imidazoline act as ligands for imidazoline receptors (IR) and the alpha-2 adrenergic receptor (α2R). IMZ, the product of the MF and MG reaction as a novel imidazolinone (IMZ) metabolite, is a potent IR and α2R antagonist that initiates a cascade of processes, including vasodilation. We therefore hypothesize that IMZ improves endothelial cell function and contributes to the therapeutic effects of MF. In the current studies we examined the in vitro effects of IMZ on endothelial cell function using HUVECs and characterized potential signaling pathways. We show that IMZ at physiological relevant concentrations induces the production of the potent endothelial derived relaxation factor (EDRF), NO, concomitant with an increase in the activation of endothelial nitric oxide synthase (eNOS). IMZ-induced NO production was blunted by pretreatment with I1R and α2R antagonists, suggesting that IMZ action is receptor mediated. We also observed that IMZ cause the activation of Akt and ERK1/2, in both a concentration and time-dependent manner. IMZ-induced activation of Akt, ERK1/2, eNOS was inhibited in the presence of a PI3K inhibitor. Interestingly, ERK1/2 phosphorylation mediated by IMZ was also reduced in the presence of Akt1/2 specific inhibitors, suggesting that ERK1/2 might lie downstream of Akt during IMZ-initiated activation. The effects of IMZ on angiogenesis function were also evaluated. In co-culture experiments, IMZ treatment significantly increased tube length compared to untreated controls. Collectively, the data demonstrate that IMZ might contribute to the protective effects of metformin on endothelial cell function by activating I1R and/or α2R-Akt-ERK1/2-eNOS-NO pathway. Studies are ongoing to further elucidate and confirm the effects of IMZ in vivo models.

1206 A Novel Metformin-Methylglyoxal Imidazoline Metabolite Sensitizes Cells to Insulin: A Potential Role in Alleviating T2DM Complications

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Reactive dicarbonyls, such as methylglyoxal (MG), are elevated in type-two diabetes mellitus (T2DM) patients. These endogenous electrophiles covalently modify proteins, which may contribute to diabetic complications. The T2DM first-line therapy, metformin (MF), significantly reduces adverse diabetic endpoints and mortality more effectively than other antihyperglycemic agents, the mechanism(s) of which remain unclear. We have identified and characterized the product of the MF and MG reaction as a novel imidazoline (IMZ) metabolite. IMZ was detected via LC/MS in MF-treated T2DM patients, and MF urinary levels directly correlated with urinary IMZ. Scavenging of MG by MF represents a possible alternative mechanism of MF drug efficacy, in addition to its antiglycogenesis properties. Imidazoline receptors (IR) are novel targets for drug development in disorders associated with T2DM because they are involved in insulin secretion/sensitization and glucose homeostasis. Thus, we examined the ability of IMZ to modulate insulin-mediated cell signaling pathways, via western blot in PC12 (express high levels of I1R and lack the α2-adrenergic receptor) and HepG2 (a common cell model for insulin signaling) cells. Combination treatment of insulin and IMZ at physiologically relevant concentrations (1 pM and 1nM) increased AKT and ERK1/2 phosphorylation above levels seen with insulin treatment alone. Moreover, IMZ also restored high glucose-induced decreases in AKT and ERK1/2 phosphorylation and AKT signaling pathway. This potentiation was not observed in the presence of I1R and α2-AR antagonists. Therefore, IMZ may enhance insulin action in the insulin-dependent AKT and ERK pathways through I1R and α2-AR activation. Preliminary in vivo pharmacokinetic studies show that IMZ (IP, 10mg/kg and 20mg/kg) is rapidly absorbed and quickly eliminated. In addition, IMZ enhanced hepatic pAKT after 40 minutes. In summary, the formation of IMZ in T2DM patients provides evidence that MF scavenges MG, potentially reducing detrimental protein modifications. This property of MF may play a role in the reduction of diabetic complications and represents a potential alternative mechanism of MF drug efficacy. Further, research in diabetic (db/db) mice are ongoing to assess whether IMZ has the ability to improve glycemic control and insulin responsiveness in vivo.

1207 In Vivo and In Silico Evaluation of Anti-diabetic Effect of White Butterfly (Clerodendrum volubile) Leaves

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White Butterfly (Clerodendrum volubile) leaf is commonly used in traditional medicine for the management of various diseases including diabetes in Nigeria. This study sought to propose the possible mechanism underlying the anti-diabetic effect of C. volubile leaves in streptozotocin (STZ)-induced diabetic rats using in vitro and in silico approach. Aqueous extract of C. volubile was prepared and its effect assessed on relevant enzymes associated with diabetes. Fifty Male Wistar rats (n=5) were randomly separated into ten groups. The induction of diabetes in rats was by a single intraperitoneal injection of STZ (65 mg/kg body weight), which C. volubile extract was administered orally to diabetic and non-diabetic animals, at the doses of 50, 100, and 200 mg/kg body weight for 14 days. Also the interaction of compounds identified from C. volubile (HPLC-DAD) on Takeda-G-protein-receptor-5 (TGR5), peroxisome proliferated activated receptor gamma (PPAR gamma) and dipeptidyl-peptidase 4 (DPP-4) was also investigated through molecular docking. Administration of C. volubile extract significantly reduced the elevated plasma glucose level and body weight, improved kidney functions, attenuated oxidative stress by decreasing MDA levels, enhancing superoxide dismutase, catalase and glutathione peroxidase activities, reinstated the lipid profile to normal level and restored pancreatic histological integrity in diabetic rats. Rutin ranked highest among all the compounds identified in C. volubile with -8.3 kcal/mol binding energy with TGR5 with Glu343 and Glu291 -7.9 kcal/mol (PPAR gamma) and -9.5 kcal/mol (DPP-4). Glu77, Arg125, Tyr240, Glu343, Tyr 662 are among residues enhancing rutin binding to these proteins. The results revealed that C. volubile possess anti-diabetic effects through the modulation of TGR5, PPAR gamma, and DPP-4. Rutin and other compound responsible for the anti-diabetic effect of C. volubile justifying the use of this plant in traditional medicine.

1208 TGF-β1 Modulates G Protein-Mediated cAMP Generation in Human Airway Smooth Muscle (HASM) Cells

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G protein coupled receptor signaling modulates human airway smooth muscle (HASM) relaxation through the β3-adrenergic receptor (β3AR/G/adenylyl cyclase axis, inducing dilation of the airways in asthma. Our previous studies showed that the pro-fibrotic cytokine, transforming growth factor beta 1 (TGF-β1), enhanced contractility of HASM. Accordingly, we hypothesize that TGF-β1 attenuates β3AR agonist-induced relaxation of HASM through modulation of G protein. HASM cells were stimulated in serum-free F12 media with TGF-β1 (10 ng/mL) overnight. Subsequently, HASM cells were treated with cholera toxin (CTX) (0.25 μg/mL; 30-60 min), the β3-agonist ISO (1 μM; 5 min), or the adenylyl cyclase direct activator, FSK (10 μM; 15 min). HASM were lysed and intracellular cAMP levels were determined by chemiluminescent immunoassay. In addition, phosphorylated myosin light chain (MLC) and total MLC were determined by immunoblot after HASM cells were stimulated with CTX (0.25 μg/mL; 30-60 min) or ISO (1 μM; 10 min) in the presence and absence of carbachol (CCh) (20 μM; 12 min) or TGF-β1. Data is represented as mean ±
Selenium plays an essential role in redox biology and redox toxicology. It is found at the active site of redox enzymes such as, glutathione peroxidases (GPx) and thioredoxin reductases (TrxR). Cell culture media is typically deficient in selenium; this deficiency could limit seleno-enzyme expression and activity, compromising the translation of data from in vitro experiments to in vivo settings. The level of selenium in cell culture media should always be a consideration. However, any supplementation must be within a range to overcome deficiency and yet not induce toxicity. The optimal level of supplementation has not been well characterized. To determine optimal levels of Se-supplementation of cell culture media we introduced varying levels of sodium selenite (Na2SeO3) or seleno-L-methionine (SLM). We established, that the window to avoid potential toxicity can be widened by using SLM instead of selenite. We assessed the expression and activity of GPx1 and GPx4 to determine the level of optimal selenium supplementation for a variety of cells. Supplementation cell culture media containing 10% FBS with 100 - 300 nM SLM, we were able to maximize GPx1 and GPx4 enzyme expression and activity. Interestingly, data from a clinical trial at The University of Iowa indicates that supplementing Se in humans does not increase GPx1 activity in blood. Subjects received ~30x the RDA (35 μg d-1) of Se as SLM daily for more than 7 mo. The level of total Se in blood increased about 50-fold. However, the activity of GPx1 in blood remained constant. Our cell culture data show the importance of supplementing cell culture media with Se to insure full activity of these enzymes. This simple maneuver will increase the rigor and reproducibility of cell culture experiments and may lead to better translation of data addressing basic redox biology.
Poisoning is a significant global and national public health problem; and the leading cause of injury death in the United States. According to the World Health Organization, an estimated 193,460 people died of intentional poisoning, and 47,478 deaths were attributed to unintentional poisoning in the United States. Approximately 60,000 emergency department (ED) visits, annually, result from unintentional medication overdoses among children under the age of 5. The purpose of this study was to examine the US trends in fatal pediatric poisonings, and to examine the recorded circumstances of fatalities from poisoning in children under 5 years old. The investigators reviewed ten years of American Association of Poison Control Centers (AAPCC) annual reports examining pediatric fatalities from years 2007–2016. Exposures to children over age five years, adults, and animals were excluded from the review. In total, there were 419 deaths in children 5 years and under, during the 10-year period. In cases where intent was known, forty percent of pediatric fatalities were due to unintentional exposures, and 14% were attributed to malicious intent. Fifty-one percent of cases were attributed to non-pharmaceutical exposures, including 20 cases of carbon monoxide poisoning, and 17 cases of disc battery ingestions. Forty-nine percent of exposures attributed to pharmaceuticals included categories such as opioids, analgesics, cough and cold preparations, antidepressants, and amphetamines and street drugs. The findings indicate an opportunity and mandate for toxicologists to participate in inter-professional collaboration for prevention measures on significant public health issues to prevent tragic poisoning deaths in young children.

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 Singapura brown ticked cats were submitted to the San Bernardino branch of CAHFS for necropsy. These animals had been found dead in an apartment along with their two deceased owners. 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 rest of the fiber. Van Kossa staining of these fibers was unrewarding. Rarely, discrete areas of hypercontraction bands were seen in individual cardiomyocytes. Based on the clinical history, gross and microscopic changes, carbon monoxide poisoning was suspected, and frozen muscle and blood from the two animals were submitted for toxicological analysis. The muscle samples were negative for cyanide by distillation method. The blood samples were analyzed for carbon monoxide by a modification of the Compac® electrochemical gas meter. The blood carboxyhemoglobin (COHb) was measured as % saturation, and values of 57 % and 41 % were found for both cats, respectively. Twenty-five percent of serum samples were positive for carbon monoxide, and the blood hemoglobin (Hb) was significantly lower in both cats, compared to the normal values for the species. The presence of CO in the blood was confirmed by gas chromatography. The results of this study suggest that CO poisoning is a significant public health issue in cats, and that routine screening for COHb should be performed in cases of unexplained death in cats. The findings of this study also highlight the importance of inter-professional collaboration for prevention measures on this significant public health issue.

Acetaminophen (paracetamol) overdose is the commonest cause of acute liver failure. Calfamafodipin (CaM) is a superoxide dismutase mimetic that prevents acetaminophen toxicity in mice. The POP Trial was a phase 1, open label, rising dose, randomised study which explored the safety and tolerability of CaM in patients with acetaminophen overdose. The POP Trial had ethical and regulatory approval. Patients were recruited in the Emergency Department of the Royal Infirmary of Edinburgh, UK, from 8 June 2017 to 10 May 2018. The inclusion criterion was: adults within 24 h of a single or staggered acetaminophen overdose that required NAC treatment. Patients were randomly assigned with a 1:1 allocation ratio to one of three sequential dosing cohorts of 8 patients (n=6 for NAC+CaM; n=2 for NAC alone). The intravenous dosages of CaM were 2, 5 and 10 µmol/kg, administered over 5 min between NAC bags. All participants experienced at least one adverse event (AE). The numbers experiencing at least 1 serious adverse event (SAE) were: NAC alone, 2/6; NAC+CaM (2 µmol/kg), 4/6; NAC+CaM (5 µmol/kg), 2/6; NAC+CaM (10 µmol/kg), 3/6. In assessing causality, there were no AEs or SAEs probably or definitely related to CaM. ALT was similar across groups. Median FLK18 rate at 20h was: NAC alone, 306 U/L (range 118-2606); NAC+CaM (2 µmol/kg), 212 U/L (98-572); NAC+CaM (5 µmol/kg), 163 U/L (100-287); NAC+CaM (10 µmol/kg), 155 U/L (103-508). The median fold increase in FLK18 (baseline to 20h) with NAC alone was 1.71 (1.24-3.57). By comparison, with NAC+CaM treatment the
fold increase in FLK18 was smaller (2μmol/kg: 1.41 (0.53-2.80); 5μmol/kg: 1.02 (0.43-1.45); 10μmol/kg: 1.07 (0.74-4.34)). The change in mir-122 across groups was comparable to FLK18. CaM was tolerated in patients treated with NAC for acetaminophen overdose and may reduce hepatocyte injury. Clinicaltrials.gov NCT03177395. Funder PledPharma AB.

1217 Delayed Treatment with 4-Methylpyrazole Protects against APAP Hepatotoxicity by Inhibition of C-Jun N-Terminal Kinase

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Acetaminophen (APAP) overdose is a major cause of hepatotoxicity and acute liver failure in the United States. N-acetylcysteine (NAC) is the only antidote available against APAP hepatotoxicity, but is only effective within a narrow therapeutic window. 4-Methylpyrazole (4MP) is a US FDA approved drug for methanol and ethylene glycol poisoning. We have previously shown that co-treatment with 4MP effectively protects against APAP-induced liver injury by inhibiting cytochrome P450-dependent metabolic activation. Since a post-treatment regimen is clinically more relevant, we tested the hypothesis that delayed treatment with 4MP is still protective against APAP hepatotoxicity and this involves novel mechanisms. Male C57BL6/J mice were treated with 300 mg/kg APAP, followed by either 50, 100 or 200mg/kg 4MP, 90 min later (when almost all APAP metabolism is completed in the mouse). Animals were then sacrificed at 2, 6, and 24 hrs and stored at -40°C. Analysis of 4MP indicated that 4MP can bind to the ATP binding site of JNK. Downstream events of JNK activation such as mitochondrial oxidant stress and the release of apoptosis-inducing factor from mitochondria were also effectively prevented. Importantly, protein adduct formation was not affected, indicating that protection from delayed 4MP treatment is independent of its effect on APAP metabolism. Further investigation revealed that 4MP treatment also promoted autophagy and the removal of protein adducts but autophagic flux experiments suggested that this is only a minor contribution to the overall protection. We conclude that delayed treatment of 4MP effectively protects against APAP hepatotoxicity through the novel mechanism of being a competitive inhibitor of JNK. Thus, 4MP can be a useful complementary antidote to NAC with different therapeutic targets.

1218 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) Dysregulation of Hepatic One Carbon Metabolism during the Progression of Steatosis to Steatohepatitis with Fibrosis in Mice

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TCDD has been linked to the development of numerous metabolic diseases including non-alcoholic fatty liver disease (NAFLD). One carbon metabolism (OCM) gene expression and metabolites levels, most notably S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH), are often altered in NAFLD. Changes in the SAM/SAH ratio affect methylation reactions and this involves novel mechanisms. Male C57BL/6 mice were treated with 0.33, 1.0, or 3.3 μg/kg TCDD (0.3-30 μg/kg) every 4 days for 8 or 28 days. At 28 days, histopathology showed increased hepatic fat accumulation, Immune cell infiltration, bile duct proliferation and collagen deposition. TCDD dose- dependently repressed adenosylhomocysteine hydrolase (AcH; EDS0 10.4 μg/kg), betaine-homocysteine S- methyltransferase (BHMT; EDS0 11.2 μg/kg), cystathionine-β-synthase (CBS), glycine N-methyltransferase (Gmmt; EDS0 11.2 μg/kg), and methionine adenosyltransferase 1A (Mat1a; EDS0 4.5 μg/kg). Accordingly, protein levels of AcH, BHMT, CBS, Gmmt and Mat1a were decreased with increased levels of betaine, homocysteic acid, and methionine, while cystathionine (cyste), dimethylglycine (dmg), and sarcosine were decreased. Absence of protective dioxin response elements (pore) within AhR enriched regions of most OCM genes following 2hrs of TCDD exposure suggests non-canonical AhR regulation. A time-course study revealed modest Mat1a and CBSexpression at 168 hrs following a single bolus dose of TCDD indicating OCM disruption maybe a delayed response. At 8 days, repression of Mat1a and AcH resulted in decreased cyst and dmg levels, and the SAM/SAH ratio. These results suggest persistent AhR activation by TCDD leads to OCM disrup-

1219 Masitinib Induces Apoptosis in Human Hepatic and Cardiac Cells

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Masitinib is a tyrosine kinase inhibitor that was temporarily approved by US FDA for veterinary use. Though masitinib is no longer approved for animal use, it is currently under phase 3 clinical trials for treating mastocytosis, rheumatoid arthritis, various types of cancers and nervous system diseases in humans. Clinically apparent acute hepatotoxicity has been associated with masitinib administration, but the mechanisms are unknown. Here we examined the short-term (6 to 24 h) cytotoxicity of masitinib in primary cultured rat, canine and human hepatocytes at concentrations normalized to human blood levels. Mode of cell death and mitochondrial functions were determined. We found that masitinib started to induce significant apoptosis and adenosine triphosphate (ATP) shortage at 4.5 μM, a concentration that equals to 1.5-fold the peak blood levels (Cmax) of masitinib in humans. At 10-fold Cmax, masitinib caused 100% lactate dehydrogenase (LDH) release in primary cultured hepatocytes, indicating that all cells were killed. Similar findings were obtained with human induced-pluripotent stem cell (iPS)-derived hepatocytes. However, in human iPS-derived cardiomyocytes (iPSC-CMs), significant cytotoxicity was not observed until the concentration was increased to 4-fold Cmax, suggesting that cardiomyocytes might be less sensitive to masitinib toxicity than hepatocytes. The apoptosis-inducing effect of masitinib was at least partially due to its capacity to induce mitochondrial cytochrome c release. These data indicate that direct hepatocyte cytotoxicity might contribute to masitinib associated hepatotoxicity.

1220 CYP2A5 Absence in PPARα-/- Mice Develop Less Obesity but More Severe NAFLD in Response to High Fat Diet

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Cytochrome P450 2A6 (CYP2A6) and its mouse ortholog CYP2A5 are mainly expressed in liver. CYP2A6 is associated with obesity and its hepatic expression is increased in patients with non-alcoholic fatty liver disease (NAFLD). High fat diet (HFD)-induced obesity and NAFLD were more severe in CYP2A5 knockout (cyp2a5-/-) mice than in wild type mice, suggesting that CYP2A5 protects against obesity and NAFLD. Very interestingly, peroxisome proliferator activated receptor α (PPARα) is upregulated in cyp2a5-/- mice. PPARα regulates lipid metabolism and glucose homeostasis in liver. To examine the possible role of PPARα in the enhanced HFD-induced obesity and NAFLD in cyp2a5-/- mice, we created CYP2A5 and PPARα double knockout (ppara/-/cyp2a5-/-) mice by crossing PPARα knockout (ppara-/-) mice with cyp2a5-/- mice. The littermate including cyp2a5-/- mice and ppara-/- mice were used as control. After 14-week HFD feeding, serum alanine transaminase (ALT) was increased in ppara-/- and cyp2a5-/- mice but not in cyp2a5-/- mice and ppara-/- mice. Haematoxylin and eosin staining in liver sections showed that hepatosteatosis was more severe in ppara-/-/cyp2a5-/- mice than in cyp2a5-/- mice and ppara-/- mice, more inflammatory foci were observed in ppara-/-/cyp2a5-/- mice than in cyp2a5-/- mice and ppara-/- mice; satellitosis (inflammatory cells surrounding big lipid droplets) and inflammation-associated nodules were observed in ppara-/-/cyp2a5-/- mice but not in cyp2a5-/- mice and ppara-/- mice. Immunohistochemistry staining showed that lipid droplet protein perilipin-2 was more extensive in ppara-/-/cyp2a5-/- mice than in cyp2a5-/- mice and ppara-/- mice. Sirius red staining and Trichrome Masson staining indicated that fibrosis was developed in ppara-/-/cyp2a5-/- mice but not in cyp2a5-/- mice and ppara-/- mice; consistently, expression of collagen and α-smooth muscle actin was increased in ppara-/-/cyp2a5-/- mice. However, body weight was increased in cyp2a5-/- mice to a greater extent than in ppara-/- mice and ppara-/-/cyp2a5-/- mice; consistently, expression of collagen and α-smooth muscle actin was increased in ppara-/-/cyp2a5-/- mice more than in cyp2a5-/- mice. Glucose tolerance test indicates that ppara-/- mice and ppara-/-/cyp2a5-/- mice were more tolerant to glucose than cyp2a5-/- mice. These results suggest that PPARα may enhance the protective effect of CYP2A5 on NAFLD but suppress the anti-obese effect of CYP2A5.
The disease burden of liver diseases is high and despite years of research much remains to be explored in the molecular mechanisms behind liver regeneration and hepatocellular carcinoma (HCC). The liver has a unique and intrinsic regenerative capacity which could be triggered by, but not limited to hepatotoxins and surgical removal of liver tissue. Several animal studies demonstrated spontaneous liver regeneration after partial hepatectomy and hepatocellular carcinoma after exposure to chemicals such as diethylnitrosamine (DEN). Interestingly, osteopontin and Wnt/β-catenin pathway independently play important roles in liver regeneration after partial hepatectomy and chemical-induced HCC in mice. Furthermore, osteopontin is a Wnt target gene. Our group previously observed a dramatic increase in the number of hepatocytes in S-phase in the transgenic mice overexpressing beta-catenin at 40 hours and 72 hours in the wild type mice following partial hepatectomy in mice. We therefore hypothesize that “Partial hepatectomy stimulate the Wnt/β-catenin pathway to potentiate liver regeneration by inducing osteopontin”. Osteopontin expression in liver tissue samples from wild type (WT), Serine-45 mutant β-catenin transgenic (TG) and β-catenin conditional knockout out mice (KO) after partial hepatectomy was assessed by immunohistochemistry. In addition, advanced imaging was performed on the stained sections. The time points post-hepatectomy considered include: 6 hours, day zero, 20 hours, 40 hours, 72 hours and 14 days. Our results showed a peak expression of osteopontin in the TG mice at 40 hours post-partial hepatectomy. In addition, there is greater expression of osteopontin in the TG mice compared to the WT mice 40 hours post-partial hepatectomy. Finally, our observation suggests that the deficiency of beta catenin suppresses the expression of osteopontin following partial hepatectomy in mice. In conclusion, Peak expression of osteopontin at 40 hours post-hepatectomy coincides with the previously observed peak proliferation of hepatocytes at the same time point in TG mice. Therefore, Wnt/β-catenin pathway may potentially regulate osteopontin expression following partial hepatectomy in mice. The interaction between Wnt/β-catenin pathway and osteopontin may play an important role in regenerative medicine and the pathogenesis of chemical-induced hepatocellular carcinoma.

Biochemical and Histological Studies on the Effect of African Iba, a Herbal Purgative, in Albino Rats

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Limited data is available about the toxicity of herbal remedies used for self-medication. Since a popular herbal remedy, African Iba Herbal Mixture (AFRIM), contains various bioactive molecules, the present study aimed to observe the biochemical and histological changes in the hepato-renal system of albino rats. Four groups of six male rats per group were used for the study. To group A was administered distilled water and served as control, while groups B, C and D were respectively administered with 100, 200 and 400 mg/kg body weight of AFRIM via gastric intubation for 14 days. Thereafter, animals were anesthetized and sacrificed by cervical dislocation; blood samples were collected for biochemical assays while the liver and kidney were processed for histopathological studies. While the administration of AFRIM did not significantly alter the levels of calcium, potassium and alanine aminotransferase across all treatment groups, aspartate aminotransferase, albumin, blood urea nitrogen were significantly (p < 0.05) reduced, especially at 200 and 400 mg/kg doses. Furthermore, there was a significant (p < 0.05) elevation in uric acid levels at the higher doses of AFRIM. Liver sections revealed mild to severe infiltration of inflammatory cells in zone 2 at 100 and 200 mg/kg of AFRIM. Kidney sections illustrated engorged glomeruli which appear shrunk with hyper-cellularity of mesangial cells at the 200 mg/kg dose. In light of this, caution and adherence to dosage prescription is strongly advised. This study is a contribution to scientific knowledge on the safety/toxicity profile of African Iba Herbal Mixture among the growing list of Nigerian herbal remedies.
Predicting drug metabolism and drug induced liver injury (DILI) remains challenging as all in vitro models, including cultured primary human hepatocytes (PHH), hepatoma cells and pluripotent stem cell (PSCs)-derived hepatocyte-like cells (HLCs) do not recapitulate many hepatic functions. We here created a robust protocol to generate PSC-hepatic progeny that significantly better approach mature PHH functions. PSC were engineered to overexpress 6 transcription factors (named HC6X). This, combined with metabolomics-based medium optimisation (named Med5), generated hepatic progeny that was gluconeogenic and used OxPhos to levels similar to PHHs. RNAseq demonstrated that the PSC-hepatic progeny were significantly more similar to PHHs than most if not all other PSC-derived hepatic cells. Among the top normalized pathways were xenobiotic metabolism, PPAR signaling, glycolysis and amino acid metabolism. An unbiased metabolomics profile of the cells is being completed. A repeated dose toxicity study with the 13 gold-standard training compounds described by the MIP-DILI Consortium (Sison-Young et al., 2017), demonstrated that HC6X-Med5 cells correctly identified 6 of 9 hepatoxins (IC50/LOEC = 10), without detecting false positive hits, which is similar to fresh PHHs, and significantly better than HepG2 or HepaRG cells. In line with their PHH-like mitochondrial activity, HC6X-Med5 cells were exquisitely sensitive to the mitochondrial toxin, Rotenone. Finally, CYP450 and UGT drug biotransformation studies demonstrated that the biotransformation capacity of HC6X-Med5 cells approximated that of the PHH-based Hepatopac system and surpassed that of HepG2 and HepaRG cells. Of note, drug toxicity and drug biotransformation were stable until at least d50 in vitro. To conclude, we developed a robust in vitro culture system consisting of genome edited PSC derived hepatic progeny cultured in optimised medium, which display drug biotransformation characteristics and accurate drug toxicity detection at levels similar to fresh PHHs, which remains stable until at least d50. This project received funding from the EU Horizon 2020 research and innovation programme, grant agreement No 681002.

Polychlorinated biphenyls have been characterized as epidermal growth factor (EGF) and transforming growth factor (TGF)-alpha secretory inhibitors in vivo. We identified a PSC hepatocytic line that can promote a fibrotic associated steatohepatitis (TASH) in exposed human populations and animal models. Current therapeutics for TASH or other subsets of NAFLD are nonexistent but are high priority due to the 25% prevalence of non-alcoholic fatty liver disease (NAFLD) worldwide. A PBC-mediated TASH animal model (C57BL/6 male mice fed a HFD) followed by epidermal growth factor (EGF) (0.2 μg/g) injections after 10 weeks post PBC exposure was conducted. Physiological liver, adipose, and serum samples were taken to measure therapeutic efficacy. Western blot, qPCR, and phosphoproteomic analyses were conducted to evaluate the mechanism of action for EGF and PBC exposure. EGF administration prevented PBC-mediated inhibition of hepatic EGF signaling. EGF also attenuated PBC-induced hepatic steatosis and bridging fibrosis, accompanied by decreased levels of steatois markers including plasminogen activator (PAI-1) and resistin. EGF administration also reduced PBC-induced elevation of hepatic free fatty acids by facilitating lipid export from the liver to the adipose tissue for storage. While EGF promoted hepatic gluconeogenesis, it however had no effect on glucose uptake. Mechanistically, EGF elicited its protective effects through EGF signaling which appeared to negatively regulate PBC-induced CAR activation. Additionally, EGF signaling positively regulated HNF4 α, a transcription factor crucial for hepatocyte function as well as LXRα and Nr2 expression and activity. Taken together, the results demonstrated that EGF administration ameliorated metabolic features caused by PBC exposures in a diet-induced obesity setting. Furthermore, EGF acted via multiple mechanisms such as phosphoregulation, nuclear receptor activity and adipokine secretion to protect mice against PBC-induced steatosis and fibrosis. These data strongly implicate the role of EGF signaling as a pharmacological target for TASH.
1229 Hepatic Fibrosis Is Induced by Ochratoxin A through SMAD Pathway in LX-2 Cells and Mice
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Ochratoxin A (OTA) is a mycotoxin produced by some species of Aspergillus and Penicillium. It occurs mainly in the process of storing cereals and spices in a high temperature and high humidity environment. OTA is the most common and toxic substance among Ochratoxins. Food product which contains OTA can cause fatal damage to kidneys and liver. This study was conducted to investigate the causal mechanism between OTA and fibrosis. We assume that liver cells exposed to OTA express TGF-β1 by its TGF-β1 receptor on the cell membrane. Then, Smad2 and Smad3 are phosphorylated in the cytoplasm and transported into the nucleus, which induces Epithelial mesenchymal transition (EMT) leading to fibrosis. LX-2 cells were treated with OTA to determine proper cell viability. With in vitro and in vivo experiment, major liver fibrosis markers such as fibronectin, E-cadherin, and α-SMA were expressed in mRNA and protein level. Especially in vivo, it is observed that OTA induces pathological damage through Masson’s Trichrome staining in mouse liver tissue. Also, the hepatic fibrosis index in serum such as albumin, ALP, ALT, AST, and bilirubin showed the degree of liver damage. The expression of TGF-β1, Smad2, and Smad3 in mRNA and protein levels is increased. These results showed the relationship between liver fibrosis by OTA and TGF-β1, and Smad pathways.

1230 Distribution of Acetaminophen Protein Adducts following Repeat Administration of Subtoxic Doses in Fed Mice
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Acetaminophen (APAP) is a widely used analgesic and is safe at therapeutic doses. APAP hepatotoxicity is initiated by formation of a reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) which depletes cellular glutathione and binds to mitochondrial proteins to form protein adducts (APAP-CYS). Though APAP-CYS adducts have been identified as biomarkers in APAP hepatotoxicity and are also the focus of a recent bioassay in plasma to measure severity of APAP overdose, it is well recognized that circulating adducts also appear after therapeautic doses of APAP, indicating that their release at lower doses is independent of liver injury. Since multiple doses of APAP are typically taken over time, especially in situations of chronic pain, we hypothesized that the development of liver injury could be dictated by accumulation of adducts over time, especially within the mitochondria. This was tested in the mouse model by repeated sub toxic doses (75mg/kg and 150mg/kg) in fed mice to reproduce clinical use of the drug, followed by measurement of adducts within the liver, mitochondria and in plasma. Since autophagy has been shown to be involved in handling of cellular adducts, the effect of their perturbation was also analyzed. Our data shows that APAP-CYS was formed in mitochondria in the absence of liver injury, though this occurred in parallel with adduct accumulation in the whole liver. Despite detectable adducts within liver and mitochondria at lower doses, release into plasma was only evident when the dose was increased, suggesting the existence of a threshold effect for plasma release of adducts. Interestingly, interference with the removal of adducts through blocking autophagy consistently increased intracellular adduct levels and exacerbated liver injury. In conclusion, repeated administration of subtoxic doses of APAP result in accumulation of APAP-CYS within the liver and mitochondria, and its appearance in plasma seems to be concentration dependent. Any interference with adduct removal through autophagy elevates addsuct levels both in mitochondria and the liver and exacerbates liver injury.

1231 Differential Activation of Hepatocyte Tissue Factor Procoagulant Activity by Necrotic and Apoptotic Cell Death

Tissue factor (TF) is the transmembrane receptor for coagulation factor VIIa and the primary activator of the coagulation cascade. The TF:VIIa complex expressed by liver parenchymal cells (i.e., hepatocytes) lacks procoagulant activity under normal conditions. However, when the liver is injured, the TF:VIIa complex initiates intrahepatic coagulation. The mechanisms required to activate TF:VIIa procoagulant function are not known. We tested the hypothesis that hepatocyte TF:VIIa procoagulant activity is increased by hepatocyte injury and functionally connected to the mode of cell death. Necrosis of primary mouse hepatocytes was induced by the toxicant acetaminophen (APAP, 0.5 mM) and c-Fos-dependent apoptosis was induced using J02 antibody (0.5 mg/ ml). APAP treatment increased outer membrane permeability consistent with necrotic cell death, indicated by release of alanine aminotransferase (ALT). A slight, but significant (~1.7 fold) increase in hepatocyte TF procoagulant activity occurred 7 hours after APAP treatment, but did not increase concomitantly to near complete cell death observed after overnight treatment. In contrast, induction of apoptosis with J02 caused a dramatic increase in TF activity prior to loss of outer membrane integrity, and this continued to increase over time. These results indicate that loss of outer membrane permeability, as in necrotic cell death, is insufficient to elicit large increases in TF procoagulant activity. By comparison, apoptotic cell death elicits rapid and robust increases in TF procoagulant activity. Overall, these results suggest that apoptotic cell death, as opposed to necrosis, is a more procoagulant form of hepatocyte cell death.

1232 Perfluorobutanesulfonic Acid (PFBS) Promotes Fat Accumulation in HepG2 Cells
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Nonalcoholic fatty liver disease (NAFLD) is one of the most prevalent chronic liver diseases. Per- and polyfluoroalkyl substances (PFASs), especially perfluorooctanesulfonic acid (PFOS), have been extensively used in food packaging, non-stick cookware, and textiles for over 50 years. However, in the past decade, a growing body of evidence has emerged, demonstrating the potential adverse effect of PFASs, including its effect on the development of NAFLD. On the other hand, there is no report investigating the effect of perfluorobutanesulfonic acid (PFBS), the major replacement for PFOS, on NAFLD. Therefore, we aimed to examine the effects of PFBS exposure on fat accumulation in HepG2 human hepatocytes. HepG2 cells were exposed to PFBS with or without 300 μM fatty acid (FA) mixture conjugated by bovine serum albumin (oleic acid:palmitic acid = 2:1) as an inducer of steatosis for 48 hours. Compared to the control, 200 μM PFBS significantly increased the triglyceride level with or without FA. FA interacted with PFBS and potentiated the fat accumulation induced by PFBS. Moreover, PFBS treatment promoted the production of reactive oxygen species (ROS) and the expression levels of genes regulating lipogenesis; acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and sterol regulatory element-binding protein 1 (SREBP1). Moreover, HepG2 treated with fatty acid uptake was also implied by the upregulation of key genes modulating the capacity of FA trafficking to liver, including peroxisome proliferator-activated receptor gamma (PPARγ) and fatty acid translocase (CD36). C/EBP homologous protein (CHOP), which is activated by endoplasmic reticulum (ER) stress, was increased after PFBS treatment. In conclusion, PFBS may promote fat accumulation in HepG2 cells by inducing lipogenesis, fatty acid uptake, oxidative stress, and ER stress. This project was supported in part by NIH R01ES028201.

1233 The Double-Hit Toxicity of Obstructive Jaundice on Liver
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Bile is crucial for the maintenance of the integrity of intestinal barrier. Obstructive jaundice (OJ) causes intestinal barrier dysfunction leading to endotoxin translocation to portal circulation due to intestinal gram-negative bacterial overgrowth and decreased tight junction protein expression. Although studies indicate a marked decrease in the hepatic glycogen content in OJ, the mechanism of this process could not be well characterized. Sixty-nine guinea pigs were randomly assigned to six experimental groups. In the OJ group common bile duct (CBD) was identified and ligated, whereas in sham operated (SO) CBD was only identified. In both groups blood glucose, insulin, glucagon and IL-1β levels were measured at 24 or 72 hours of postoperative period, while oxidative stress in liver and terminal ileum was determined by the levels of nitric oxide, reduced glutathione (GSH) and malondialdehyde. The results of glucose consumption rate were expressed in percentage per minute. Histological assessment of cholangitis, necrosis, inflammation and quantification of hepatocyte necrosis were made at 24 and 72-hour following OJ. Intestinal mucosal barrier dysfunction was documented by subepithelial edema, sporadic mucosal denudation and portal endotoxin translocation. Excessive endotoxin exposure via portal circulation creating first-hit toxicity in hepatocytes stimulated the production of IL-1β, nitric oxide and superoxide anions in the liver, provoking focal hepatic necrosis. Thereby, biliary obstruction was accompanied by increased levels of lipid peroxidation in intestinal and hepatic tissues as well as the depletion of GSH. Second hit toxicity due to excessive bile acid accumulation in liver provoked the hepatic fibro-
sis, while inducing G protein-coupled cell surface receptor (TGR5)-mediated cholangiocyte proliferation, as well as alterations in pancreas. After first-hit related IL-1β expression, bile acid-induced proliferation of cholangiocyte, and protection of cholangiocytes from apoptosis indicated the increased expression of TGR5 in cholangiocytes, in response to bile acids. Seven-fold increase in serum glucagon levels and 2.8-fold higher insulin under normoglycemic conditions confirmed the TGR5 activation and its effect on glucagon-GLP-1 pathway.

1234 Acute Liver Injury Drives Fibrin(ogen) Cross-Linking Independent of Thrombin-Catalyzed Fibrin Polymerization

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Acute liver injury following acetaminophen (APAP) overdose is associated with activation of the blood coagulation cascade and deposition of fibrinogen in the liver. We previously documented that hepatic fibrinogen deposition is central to promoting repair of the APAP-injured liver. The traditional view of blood coagulation presents an entrenched hypothesis that fibrinogen deposition is driven by a canonical pathway wherein thrombin-catalyzed fibrin polymer formation is a prerequisite for fibrin cross-linking by the transglutaminase factor XIII (FXIII). However, a lack of specific tools to differentiate the functions of soluble fibrinogen and fibrin polymer in vivo has left this hypothesis largely untested. To directly address this longstanding assumption, we analyzed fibrinogen (Fib465m) which expresses normal levels of soluble fibrinogen incapable of thrombin-mediated fibrin polymer formation (i.e., a form of the molecule locked in the soluble form), following acute liver injury with APAP overdose (300 mg/kg). APAP-challenged Fib465m mice displayed a reduction in plasma fibrinogen and increased hepatic fibrinogen deposition that was identical to APAP-challenged wild-type (WT) mice. Moreover, hepatic levels of high-molecular-weight cross-linked fibrinogen were identical in Fib465m mice and WT mice after APAP challenge. Comparably, APAP challenge significantly reduced plasma fibrinogen in both WT and FXIII–/– mice, but fibrinogen cross-linking was significantly reduced in livers of APAP-challenged FXIII−/− mice compared to APAP-treated WT mice. These results offer the first evidence that thrombin-mediated polymerization is in fact not required for fibrinogen (cross-linking in vivo). Rather, the deposition of fibrinogen in the APAP-injured liver appears to occur by a mechanism distinct from traditional blood coagulation that occurs within injured vessels.

1235 High-Throughput Screening for Evaluating Drug-Induced Inhibition of Bile Acid Transporters Using Humanized Hepatocytes Generated from Chimeric Mice


Cholestasis which results from inhibition of hepatic bile-acid efflux transporters has been proposed to play a role in drug-induced liver injury (DILI), one of the most frequent safety-related reasons for drug attrition and withdrawal. To better understand risks associated with cholestasis, we have developed a high-throughput cell-based in vitro assay which can be used during different phases of drug discovery. We firstly compared two candidate cell sources; PBX-cells, freshly isolated human hepatocytes from humanized mouse liver, and HepaRG cell line in terms of gene expression, membrane polarity, and response to cyclosporine. A. PBX-cells showed more transcriptomic, structural and functional characteristics, and provide a capability for high throughput screening and a robust and consistent drug response. Fluorescent bile acid derivative cholyl-L-lysyl-fluorescein (CLF) was used to quantify drug-induced efflux transport inhibition in these hepatocytes. Accumulation of CLF in apical bile-canicular lumen was inhibited by cyclosporine A in a dose-dependent manner. Forty-five pharmaceutical compounds with or without cholestatic potential were examined to investigate the predictive power of the assay system. Over 90% of the compounds (21 of 23) with cholestatic potential exhibited safety margin (IC100/IC90) > 35, resulting in sensitivity of 91.3%, specificity of 86.4%, and a balanced accuracy of 88.9%. Our assay system demonstrated increased predictive power than industry standards cell-free bile salt export pump (BSEP) membrane vesicle assay and rat hepatocyte-based CLF efflux assay, possibly by the potential involvement of other efflux transporters, metabolic pathways and/or species differences. The combination of high-throughput CLF efflux and the 3D liver spherical cytotoxicity assays enhanced DILI risk predictability. Our CLF efflux assay can be used as a surrogate for BSEP MVA. When it is combined with other cytotoxicity endpoints, it can also improve drug candidate prioritization.

1236 Exposures to Polychlorinated Biphenyls Altered the Hepatic Proteome

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Polychlorinated biphenyls (PCBs) are associated with non-alcoholic fatty liver disease (NAFLD) in human subjects and animal models. Based on their structure and ability to activate the aryl hydrocarbon receptor, PCBs are classified as: dioxin-like (DL PCBs) and non-dioxin-like (NDL PCBs). Both types of PCBs are known to disrupt hepatic metabolism and modulate NAFLD; however, the exact mechanisms are still unclear. In the study, PCB mechanisms were investigated using transcription factor analysis of proteomics data in a diet-induced obesity mouse model of NAFLD exposed to both DL and NDL PCBs. Mice were fed a 42% fat diet and exposed to low-dose PCBs by gavage, PCB exposure included Aroclor1260 (20 mg/kg, a DL PCB mixture); PCB126 (20 μg/kg, a DL PCB congener); or Aroclor1260 (20 mg/kg)+PCB126 (20 μg/kg). After 12 weeks, the mice were phenotyped and hepatic proteomics was performed (LC/MS/MS following TMT metabolic labeling and reverse high pH separation of peptides). Spectra were matched to mouse protein database. PEAKS Studio 8 software quantitated the normalized abundance of select peptides. 2-way ANOVA was performed using R software and transcription factor analysis (TFA) was performed using MetaCore. Aroclor1260 mediated a transition from diet-induced steatosis to steatohepatitis; and PCB126 prevented this progression. PCB126 increased mice. PCB 126 reduced free fatty acids and decreased serum lipids. 8,355 hepatic proteins were identified by proteomic analysis. Protein abundance was altered by Aroclor1260 (190 proteins); PCB126 (1529 proteins); and the interaction of Aroclor1260 with PCB126 (87 proteins). TFA predicted the following alterations in transcription factor function: Aroclor1260-Hnf4α, Erry, and 5 others; PCB 126-Hnf4α, Lxrα/β, Srebp1/2 and 7 others including Nf-kb1 and CREB1; interaction of Aroclor 1260 and PCB 126-Hnf4α, Erra, and 3 others. PCB exposures differentially modulated diet-induced NAFLD and the hepatic proteome. PCB 126 altered 18.3% of all identified liver proteins. Importantly, these effects occurred 12 weeks after a single low-dose exposure.

1237 Restoration of Cellular Circadian Rhythm by FGF15/19 in Prevention of Non-Alcoholic Fatty Liver Disease

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Long-term disruption of circadian rhythm by shift-work, jet-lag, and light-at-night is associated with increased risk of metabolic syndrome, including non-alcoholic fatty liver disease (NAFLD) in both animals and humans. Recent studies indicate that SIRT1 deacetylase plays an important role in maintenance of hepatic homeostasis by linking hepatic metabolism to circadian rhythm. Fibrolast growth factor 15 (FGF15), the mouse homologue of human FGF19, is an endocrine FGF secreted from intestines responding to increased bile acid. It is critical in suppressing bile acid synthesis and improving insulin sensitivity. Direct in vivo evidence demonstrated that FGF15 play an important role in stimulating the phases of priming and termination of liver regeneration. To investigate the molecular mechanism underlying the modulation of liver regeneration by FGF15, we compared NAD+ level and SIRT1 activity as well as mRNA expression levels of genes involved in circadian rhythm and lipid metabolism in liver tissues collected from diet-induced NAFLD and the hepatic proteome. PCB26 increased liver free fatty acids and decreased serum lipids. 8,355 hepatic proteins were identified by proteomic analysis. Protein abundance was altered by Aroclor1260 (190 proteins); PCB126 (1529 proteins); and the interaction of Aroclor1260 with PCB126 (87 proteins). TFA predicted the following alterations in transcription factor function: Aroclor1260-Hnf4α, Erry, and 5 others; PCB 126-Hnf4α, Lxrα/β, Srebp1/2 and 7 others including Nf-kb1 and CREB1; interaction of Aroclor 1260 and PCB 126-Hnf4α, Erra, and 3 others. PCB exposures differentially modulated diet-induced NAFLD and the hepatic proteome. PCB 126 altered 18.3% of all identified liver proteins. Importantly, these effects occurred 12 weeks after a single low-dose exposure.
The Role of LCN2 in Acetaminophen-Induced Acute Liver Failure

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Acetaminophen (APAP) overdose is the most common cause of Acute Liver Failure (ALF) in the US. Following APAP overdose, toxicity is initiated by the conversion of APAP to its reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which binds covalently to mitochondrial proteins leading to mitochondrial necrosis and sterile inflammation. Lipocalin-2 (LCN2), is an acute-phase innate immune protein, upregulated during tissue injury in various organs including the liver. Recent data from our lab indicates that LCN2 KO mice are protected against ALF caused by APAP overdose compared to WT mice. This protection is not due to decreased bioactivation based initial injury, but due to differences in the progressive phase of liver injury at later time points (24-48hr). LCN2 KO mice exhibit significantly less hepatocellular damage (ALT and histopathology) and higher liver regeneration response post APAP overdose. However, the mechanism of LCN2’s involvement in ALF resulting from APAP overdose remains ambiguous and is the objective of the present study. During APAP-induced necrosis, hepatocytes release Damage Associated Molecular Patterns, of which is High Mobility Group Box 1 (HMGB1). HMGB1 can have lipopolysaccharide (LPS) like properties, playing a role based on its location in the nucleus, cytoplasm, or extracellularly. Cytoplasmic HMGB1 binding to Beclin-1 induces a favorable autophagic response in support of hepatocyte regeneration. We hypothesize that causative relationships between IL-6-HMGB1, Beclin-1, and LCN2 play a role in mediating progression of injury. Pro- and anti-inflammatory cytokine expression in WT and LCN2 KO were assessed by ELISA and immunoprecipitation, respectively, over a time course. IL-10 is not differentially expressed between the two groups negating its role in the observed protection. We observed significantly higher IL-6 expression at 0, 24, 36, 48hr in LCN2 KO mice as compared to WT. This corroborates with IL-6’s role in promoting liver regeneration and higher PCRNA in LCN2 KO. In our model observed previously. Ongoing studies investigate the extent of HMGB1 and Beclin-1 binding between LCN2 KO and WT mice as a potential mechanism for progression of injury. Findings from this project will identify a novel pathway involving LCN2 and potentially recognize targets that attenuate/prevent the progression of liver injury leading to ALF in APAP overdose cases.

Insulin Action and Steatosis Can Be Evaluated with Bioluminescent Metabolite Detection Assays

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Metabolic syndrome is a cluster of conditions - including high blood pressure, central obesity, elevated blood sugar, and high levels of triglycerides and cholesterol - that when occurring together, increase one’s risk for diabetes and cardiovascular disease. Two factors connecting these conditions are the action of insulin and the accumulation of lipids (i.e. steatosis). Insulin stimulates glucose uptake and suppresses lipolysis, and inhibits gluconeogenesis. In contrast, insulin resistance leads to increased gluconeogenesis, decreased glucose uptake, and steatosis. Although not inherently harmful, when steatosis occurs for extended periods of time (e.g. NAFLD or NASH), it can lead to chronic inflammation, cirrhosis, and potentially carcinoma. We demonstrate that core luminescent technology that couples specific metabolite dehydrogenases to the production of NAD(P)H and the generation of light can be applied in cellular models related to metabolic syndrome, reducing the need for animal studies. Data from a panel of assays including glucose uptake, glycerol detection and triglyceride detection demonstrate the relevance of the in vitro models. The glucose uptake assay can measure > 5-fold increases in cell surface glucose transporters upon stimulation of 3T3-L1 MBX adipocytes with 1 uM insulin. We observe a 4-fold inhibition of gluconeogenesis in liver microtissues by 10 nM insulin with the glucose detection assay. As measured with the glycerol detection assay, 3T3-L1 MBX adipocytes exhibit a 4-fold increase in lipolysis upon stimulation with 25 nM isoproterenol, which can be suppressed 2-fold by 150 nM insulin. Moreover, using HepG2 cells as a model in NAFLD, we observe a 5-fold increase in intracellular triglyceride upon overnight incubation with 0.3 mM BSA-bound fatty acids as measured by the triglyceride detection assay.
Hepatocyte Injury Drives Atypical Fibrinogen Cross-Linking: Potential Role of TGM2

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Acetaminophen (APAP)-induced liver injury is associated with activation of the blood coagulation cascade and accumulation of the clotting protein fibrinogen in areas of hepatocellular necrosis. Prior studies have suggested that hepatocyte-associated tissue transglutaminase (TGM2) can cross-link fibrinogen independent of coagulation reactions, but the impact of APAP cytotoxicity on this process has not been investigated. We determined the role of TGM2 in hepatocyte-catalyzed fibrinogen cross-linking and tested the hypothesis that APAP-induced hepatocyte cytotoxicity increases fibrinogen cross-linking. Primary hepatocytes were isolated from wild-type mice and treated overnight with various concentrations of APAP (0-5 mM) in serum free Williams’ Medium E. APAP caused a concentration-dependent increase in hepatocyte necrosis, as determined by increased release of alanine aminotransferase into the cell culture medium. Additionally, levels of cross-linked fibrinogen protein complexes, measured by capillary western blotting, were higher in APAP-killed hepatocytes compared to untreated hepatocytes, suggesting that 1) primary mouse hepatocytes express fibrinogen in culture and 2) APAP cytotoxicity increases cell-associated fibrinogen cross-linking activity. Similar results were obtained in preliminary studies in which hepatocytes were killed in the presence of exogenous human fibrinogen (10 µg/ml); cross-linked fibrinogen levels were increased in APAP-killed hepatocytes, and exogenous fibrinogen did not alter APAP cytotoxicity. Interestingly, levels of cross-linked fibrinogen were significantly reduced in hepatocytes isolated from TGM2-/- mice compared to wild-type mice, implying that hepatocyte-associated TGM2 can cross-link fibrinogen. Overall, the results suggest that APAP-induced hepatocyte necrosis increases direct TGM2-catalyzed fibrinogen cross-linking, implying a novel mechanism whereby fibrinogen structure may change during liver injury.

Sitagliptin Exacerbates Hepatic Inflammation and Necrosis in Rats Fed High Cholesterol Diet

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Western diets are comprised of meat, poultry and dairy products, all of which are rich in cholesterol (Cho). High Cho is associated with the development of a proinflammatory state and is a documented risk factor for atherosclerosis, nonalcoholic fatty liver disease and progression to a more severe inflammatory disease called nonalcoholic steatohepatitis. Sitagliptin, a dipeptidyl peptidase-4 inhibitor, is an oral anti-diabetic drug. Independent of its glucose lowering effects, sitagliptin confers anti-inflammatory effects. Therefore, studies were conducted to investigate the effects of sitagliptin on hepatic inflammation in rats fed a high Cho diet. Adult male Sprague Dawley rats were fed a control or high Cho diet. Rats on each diet were gavaged with either vehicle or sitagliptin (100mg/kg/d) till the end of the experiment. On day 36 the rats were euthanized, and livers were harvested for analysis of triglycerides, oxidative stress and fibrotic markers by biochemical methods and histopathological evaluation by H&E staining. Hepatic lipid accumulation was assessed by oil red O and fibrosis by picrosirius staining methods. Rats on high Cho diet developed fatty liver with increased lipid accumulation which was unaffected by sitagliptin. Cho diet slightly increased hepatic expression of inflammatory stress genes (Nos2, Lox1, Inos), and surprisingly, sitagliptin exacerbated the mRNA expression of each of these genes. The expression of liver fibrin(ogen) markers (Tgfβ, aSmα, Mmp9, Timp1, Tlr4) was also increased by sitagliptin in high Cho group. Additionally, the presence of sporadic fibrosis was confirmed in Cho-sitagliptin group. However, the inclusion of Met to the high Cho diet resulted in significant reversal of markers of hepatic oxidative stress, lipid accumulation, and fibrosis compared to the rats on high Cho diet receiving sitagliptin. Our data indicate that sitagliptin in conjunction with high Cho diet intensifies the oxidative damage resulting in NASH like symptoms. Surprisingly, the negative effects of high Cho and sitagliptin were at least partially reversed by the inclusion of high Met in the diet.

Direct Activation of Tissue Factor:Factor VIIa Procoagulant Activity by Bile Acids

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Tissue factor (TF) is the transmembrane receptor for the plasma coagulation factor VIIa (FVIIa), and the TF:FVIIa complex is the primary activator of the blood coagulation cascade. We posited that post-translational regulation of TF:FVIIa activity is critical for the prevention of excessive coagulation in the normal liver, where FVIIa present in plasma has unrestricted access to the TF-expressing hepatocytes. Hepatocyte-associated TF:FVIIa activates coagulation during liver disease, but the mechanisms controlling hepatocyte TF:FVIIa procoagulant activity in the liver are not known. Coagulation occurs rapidly in settings of liver damage where plasma bile acids are increased, and we found that bile acids significantly increased procoagulant activity of TF:FVIIa in cultured primary hepatocytes. However, the exact mechanism whereby bile acids increase procoagulant activity of the TF:FVIIa complex is not known. We tested the hypothesis that bile acids directly increase the procoagulant activity of the TF:FVIIa complex. Recombinant full-length human TF (10 nM) was mixed with human FVIIa (5 pM) to form the TF:FVIIa complex. Various pathologically relevant concentrations (0-500 µM) of the bile acid, sodium glycochenodeoxycholate (GCDCA) were added, and the TF:FVIIa complex activity was determined by measuring the conversion of coagulation factor X (FXa) to activated FXa using a chromogenic substrate. The TF:FVIIa complex alone had little procoagulant activity, however, the addition of GCDCA evoked a robust and concentration-dependent increase in TF:FVIIa procoagulant activity, indicating by significantly increased FXa generation. The results indicate that bile acids directly increase TF:FVIIa procoagulant activity, suggesting that bile acids may be an important trigger of TF:FVIIa procoagulant activity in the context of certain liver diseases.
Dysregulation of hepatic circadian rhythmicity is associated with the development of metabolic disorders such as non-alcoholic fatty liver disease (NAFLD). Aryl hydrocarbon receptor (AhR) activation alters the hepatic expression of several core clock regulators, however the impact on circadian-controlled hepatic metabolism and the underlying mechanisms responsible have not been elucidated. This study examines the effects of AhR activation on hepatic transcription and metabolome rhythmicity in male C57BL/6 mice orally gavaged with 30 μg/kg 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) every 4 days for 28 days. Hepatic RNA-Seq analysis detected diminished rhythmicity in 15 core clock regulators (e.g. Arntl, Clock, Nr1d1, Per1, Per2, Nfil3), involving either a ≥3.3-fold suppression in amplitude or complete loss of oscillation. Accordingly, protein levels (Arntl, Rev-Erbα, Nfil3) and genomic binding (Arntl) of select regulators were reduced and arhythmic following treatment. Chromatin immunoprecipitation (ChIP) assays demonstrated co-binding of AhR, Arntl, and Clock at E-box response elements within Per1, Per2, and Nr1d1, suggesting AhR binding may directly interfere with ARNTL-CLOCK transcriptional regulation of target genes. Consequently, the oscillating expression of 99.6% of 5,636 clock-controlled hepatic genes was abolished including genes associated with lipid metabolism, glucose/glycogen metabolism, bile acid homeostasis, heme biosynthesis, and redox homeostasis. For example, TCDD abolished the rhythmic expression of the rate-limiting enzymes in both gluconeogenesis (Pck1) and glycogenesis (Gys2), consistent with the depletion and loss of rhythmicity in hepatic glycogen levels. Examination of polar hepatic extracts by targeted mass spectrometry revealed that virtually all oscillating metabolites lost rhythmicity following treatment. Collectively, these results suggest TCDD decoupled hepatic metabolism from feeding/fasting cycles, altering metabolic efficiency and energy storage.
Most idiosyncratic drug-induced liver injury (IDILI) appears to result from an adaptive immune attack on the liver. Recent evidence suggests that the T-cell response may be facilitated by the loss of immune tolerance. In this study, we explored the hypothesis that hepatocyte-derived exosomes (HDEs) are important for maintaining normal liver immune tolerance. ExoQuick-TC™ was used to enrich exosomes from the conditioned medium of primary human hepatocytes (N=5 donors) or from a medium that was not hepatocyte exposed (mock control). THP-1 monocytes were then treated with HDEs or an equivalent volume of mock control for 24 h, followed by a 6 h stimulation with LPS. HDEs induced a significant decrease in the LPS-induced media levels of interleukin-8 (IL-8) and monocyte chemotractant-1 (MCP-1) (p<0.0001). A trend toward a decrease in tumor necrosis factor alpha (TNF-α) was also observed. Gene expression profiling performed in THP-1 cells just prior to LPS-induced stimulation identified 72 significantly differentially expressed genes in the exosome-exposed monocytes (p<0.05 with fold change ≥ 2.25), all of which were decreased compared to mock control. Pathway enrichment analysis of these genes revealed HDE-induced downregulation of the innate immune response. The greatest alteration was observed in C-C motif chemokine ligand 3 (CCL3) mRNA, with an ~6.5-fold reduction (p=0.001). MicroRNA (miRNA) profiling was performed on the HDEs collected from 4 donors to identify exosome contents that may drive immune suppression. We observed a strong concordance among predicted miRNA target genes for the 50 most abundant miRNAs in HDEs and the differentially expressed genes observed in the THP-1 cells. Two miRNAs predicted to downregulate CCL3 (miR-149-3p and miR-24-3p) were among the 50 most abundant miRNAs in the HDEs. Taken together, our data suggest that HDEs play a role in maintaining normal liver immune tolerance. Future experiments will explore the possibility that IDILI drugs promote the loss of homeostatic HDE signaling.

**1255 Gasoline-Induced Oxidative Stress in Rats**


Gasoline, having benzene as one its aromatic hydrocarbon compounds, is a ubiquitous environmental pollutant that has been implicated in cellular toxicity, via induction of oxidative stress which results in various cytopathological alterations. This calls for an urgent search for cheaper and potent natural antioxidants. A notable source of exogenous antioxidants (capable of enhancing the ameliorative role of endogenous antioxidants) is Moringa oleifera leaf. Therefore, this study was carried out to evaluate the ameliorative effects of methanolic extract of Moringa oleifera leaf (MEMOF) on gasoline-induced oxidative stress in rats using selected antioxidant parameters and liver function indices. Twenty four (24) male and female albino rats (Rattus norvegicus) of weights ranging from 150-170 g were randomly divided into four groups of six rats each: Group I rats were orally administered 100 mg/kg body weight of MEMOF; Group II rats were exposed to gasoline while Group IV rats were exposed to gasoline and orally administered 100 mg/kg body weight of MEMOF; Group III rats were exposed to gasoline while Group IV rats were exposed to gasoline and orally administered 100 mg/kg body weight of MEMOF. Exposure period of 8 hours per day was adopted for 12 weeks and the route of exposure was inhalation. After the period of administration, various liver and plasma parameters were determined. Subchronic gasoline fume exposure significantly (p<0.05) elevated plasma phenol, malondialdehyde and oxidative DNA damage product (BOH₂dG) levels in rats compared to control. These alterations were significantly reversed (p<0.05) following oral administration of MEMOF. The levels of liver antioxidant parameters (zinc, selenium, magnesium, glutathione, catalase, superoxide dismutase) which were significantly reduced (p<0.05) by gasoline exposure compared to control were significantly reversed (p<0.05) by MEMOF. The plasma activities of alanine aminotransferase, aspartate aminotransferase, gamma glutamyltransferase as well as the total bilirubin and total protein concentrations were significantly (p<0.05) elevated following exposure to gasoline compared to control; however, this was also significantly reversed (p<0.05) by MEMOF. Histopathological analysis of the liver, following exposure to gasoline, also revealed loss of normal structural architecture compared to control; however, this was ameliorated by MEMOF. These results revealed that MEMOF possesses hepatoprotective activity against gasoline-induced oxidative stress in rats; thus, it may exhibit the same activity in subjects exposed to gasoline.
Interspecies differences have limited the predictive utility of hepatic toxicity studies performed using standard rodent models. Differences in cytochrome P450 enzymes and function can lead to erroneous conclusions on the safety, or lack thereof, for human use. Therefore, it is important to develop and validate animal models to better identify drugs that may be hepatotoxic, and allow those that do not pose a risk to continue in development. The goal of this study was to evaluate a chimera mouse developed to have a humanized liver to determine if this model can demonstrate human-specific hepatotoxicity in vivo as compared to non-humanized and genetically humanized mouse models. These studies tested fluoxetine (10 mg/kg, IP), flutamide (100 mg/kg, PO) and trovafloxacin (50 mg/kg, PO), that are approved drugs with reported cases of hepatotoxicity not predicted by rodent studies. Five mice per group, using hepatic humanized TK-NOG, control TK-NOG, human CYP3A4 or CYP2D6 knock-in, and C57Bl/6 strains, for a total of 20 mice per drug, were dose for daily as 28 days. Mouse weights and survival were monitored and blood samples taken weekly to evaluate drug levels. At study end we evaluated CBC/serum chemistry, leukocyte subpopulations via flow cytometry and histopathology. Hepatic humanized mice treated with fluoxetine showed decreased survival (2/5 died before day 28) along with decreased hematocrit, and elevated total bilirubin level, ALP and ALT as compared to other groups. Trovafloxacin treated hepatic humanized mice also had early study death (4/5 died before day 28) as compared to the other mouse strains. They also showed reduced leukocyte counts and increased total bilirubin level compared to other strains. All hepatic humanized mice treated with flutamide developed severe illness at day 7 and required euthanasia while the other strains were unaffected. Flutamide treated hepatic humanized mice showed increased ALP levels compared to other strains. These results suggest that hepatic-humanized TK-NOG mice, but not other strains, developed clinical and serologic evidence of hepatotoxicity when treated with fluoxetine, trovafloxacin, or flutamide and demonstrate that hepatic-humanized mice may improve our ability to detect drug-induced liver injury and enhance pharmaceutical safety in pre-clinical and post-marketing phases of development.
Identification of Candidate Risk Factor Genes for Human Idelalisib Toxicity Using a Collaborative Cross Approach

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Idelalisib is a phosphatidylinositol 3-kinase inhibitor highly selective for the delta isomorph that has shown good efficacy in treating some hematologic malignancies. Rare, but potentially serious liver and lung toxicities were associated with idelalisib use in clinical trials. In this study, the Collaborative Cross mouse population was utilized to identify genetic factors associated with the drug response that may inform risk management strategies for idelalisib in humans. Eight (8) male mice from 50 Collaborative Cross strains were treated daily for 14 days by oral (gavage) with either vehicle (0.5 % w/v carboxymethylcellulose and 0.1% w/v Tween 80) or idelalisib at a dose selected to achieve human-relevant exposures (150 mg/kg/day). Idelalisib was well tolerated across all strains and no idelalisib-related clinical observations were observed. Strain-dependent differences in drug concentration were observed in plasma samples collected at the approximate T_max on study Days 1, 7, and 14 (p<0.001 for strain, one-way ANOVA). While no overt liver injury was observed, suggestive treatment-induced alterations in plasma total bile acids (p=0.1550) and microRNA-122 (p=0.0982 for treatment, two-way RM ANOVA) were observed and may indicate early hepatocellular stress required for immune-mediated hepatotoxicity in humans. Small but statistically significant elevations in the total cell count of terminal bronchioalveolar lavage fluid were also observed in response to idelalisib treatment (p=0.0122 for treatment, two-way RM ANOVA), which may be analogous to pneumonia observed in the clinic. Genetic mapping identified loci associated with interstrain idelalisib concentration and the other three treatment-related endpoints in Collaborative Cross mice: total bile acids, terminal plasma microRNA-122, and bronchoalveolar lavage fluid cell count, corrected for modest correlations with drug concentration (r<0.025, Pearson’s correlation). Thirteen (13) priority candidate quantitative trait genes identified in mice may now guide interrogation of risk factors for adverse drug responses associated with idelalisib in humans.

Characterization of GCDC Transport by Human Hepatic Uptake Transporters for In Vitro Testing Purposes

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Bile acids and bile salts (BAs/BSs) contribute in several physiological processes including signaling pathways, absorption of fat, or elimination of cholesterol. Primary bile acids and their conjugates are formed in the hepatocytes, then excreted into the bile. Bile is depleted in the intestine where bacterial deoxyxilation and unconjugation occur, and the majority of the bile salt species gets reabsorbed into the blood and circulates back to the liver. Giving their detergent nature, high concentration of BAs/BSs intracellularly or in the circulation system can lead to cytoxity. Therefore, testing the effect of drug candidates with high hepatic clearance on the transport of BAs/BSs is an issue of critical importance. The most commonly used probe substrate in in vitro test systems is taurocholate (TC), although the concentration of taurine-conjugated bile salts in human is two/three-fold lower than that of glycine-conjugated species, whereas taurine conjugation is the main modification in rats. Using glycochenodeoxycholate (GCDC), one of the most relevant conjugated bile salt in human, as probe substrate in in vitro test systems might provide better prediction on the effect on enterohepatic circulation of bile salts. HEK293 cells transduced with OATP1B1 and OATP1B3, as well as NTCP expressing CHO and HEK293 cells were used in uptake assay format. Proof of concept (POC) experiments were carried out with radiolabeled TC and unlabeled GCDC, sulfated GCDC and chenodeoxycholate-sulfate (3S-CDC) at two concentrations and two timepoints. All bile salts were transported by OATP1B1 and 1B3 in a time- and concentration-dependent manner, while only TC and GCDC were picked up as substrates for NTCP. Since GCDC was efficiently transported by all transporters, full transport characterization on OATP1B1, 1B3 and NTCP was conducted with tritiated TC and GCDC as probe substrates. Michaels-Menten constants (Km) were around three-fold lower for GCDC than TC, showing higher affinity for that bile salt. Inhibitory effect of known substrates and inhibitors (atorvastatin, CCK8, diclofenac, pravastatin, telmisartan and troglitazone) on both probes was also tested. Based on the obtained data, and the higher in vivo relevance, the authors suggest replacing TC with GCDC for human in vitro test systems.

Assessing Drug-Drug Interactions Using the HEPATOPAC Model, a Long-Term In Vitro Liver Platform

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Accurate prediction of drug-drug interactions (DDI) using in vitro models is critical in drug development as these studies ultimately influence the design of clinical trials. Current in vitro models lack the full complement of drug metabolizing enzymes and do not reflect the biochemical mechanisms present in hepatocytes in vivo. Also, these models are short-term and cannot recapitulate the effects of long-term drug exposure such as metabolism of enzyme inhibitors or enzyme re-synthesis after inhibition. The HEPATOPAC model, a long-lived, metabolically stable model is ideally suited to address these limitations. To this end, human HEPATOPAC cultures were treated with varying concentrations of selective CYP3A4 inhibitors, ketoconazole (0.1µM-10µM) or verapamil (1µM-30µM), for varying time intervals (1.5hrs-48hrs). The results showed a concentration-dependent decrease in CYP3A4 enzyme activity. Additionally, at low micromolar concentrations, there was a trend towards increasing CYP3A4 activity over time suggesting that the enzyme was re-synthesized during that period. Moreover, MS analyses showed a time-dependent decrease in inhibitor concentration (except at the maximum test concentrations), indicating that the inhibitors were being metabolized. Further studies seek to use HEPATOPAC cultures to determine the changes in AUC and IC50 values for drug-drug interaction between midazolam (a CYP3A4 substrate) and ketoconazole or verapamil. These preliminary findings provide evidence of CYP3A4 re-synthesis and inhibitor metabolism, and hence, the value of the HEPATOPAC model as a tool for accurate in vitro assessment of drug-drug interactions.

In Vitro Evaluation of Hepatotoxicity by Amiodarone in Micropatterned Cocultured Hepatocytes (HEPATOPAC) Using Liver-Specific Biomarkers

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Drug induced liver toxicity (DILI) is a major reason for discontinuing drug development programs and identifying potential risk for DILI is therefore important. Several in vitro tests have been developed to assess hepatotoxicity risk. Models using polarized primary hepatocytes with functional drug metabolizing enzymes and transporters, such as sandwich cultured hepatocytes, synthetic liver-on-a-chip models, or in vitro cocultured hepatocytes and micropatterned cocultured hepatocytes (HEPATOPAC) are used because their in vivo-like properties are considered to be among the more physiologically relevant models. A drawback of many in vitro tools however is lab-to-lab variability. Micropatterned co-cultures (HEPATOPAC) have a tightly controlled architecture and because of this we hypothesized its lab-to-lab variability might be minimal. In addition, the model has a life-span of several weeks in culture, allowing repeat dosing of test compounds. We therefore evaluated the known hepatotoxicant amiodarone in HEPATOPAC and compared results to published data. Using albumin, urea, adenosine triphosphate (ATP) and glutathione (GSH), we found TC50 (the concentration that decreases a response by 50%) values close to published values. After single dosing, similar TC50 values were obtained on day 5 and 9 using intracellular ATP and GSH levels. After dosing every 2 days, we measured TC50 values of increasing potency over time, using albumin and urea levels in the medium. No toxicity was observed for negative control acetysalicilic acid (aspirin) at any of the conditions. In conclusion, we successfully used the HEPATOPAC toxicity assay in our lab and demonstrated low variability between our and published data, indicating the robustness of this method between labs.
3D liver spheroids made from primary human hepatocytes (PHHs) are being adapted to in vitro systems for drug discovery and development activities such as liver safety assessment and disease modeling. Characterization, validation and standardization of liver spheroid culture are some key aspects to consider in order to successfully implement these novel 3D liver model-based assays. Because of large lot-to-lot variations among PHH lots, qualifying hepatocytes for liver spheroid culture could significantly facilitate the researcher’s efforts to standardize the use of this novel 3D liver spheroid model. In this study, we have performed qualification studies for multiple lots of PHHs for liver spheroid culture using a standard protocol in Corning Ultra-Low Attachment Spheroid Microplates. Liver spheroid formation, stable morphology and viability in long term culture (up to 4 weeks) are main criteria used for these qualification work. Characterizations for liver functions such as albumin secretion, drug metabolizing enzyme activities, cytotoxicity response are shown to demonstrate the possible utilization of liver spheroids made from these spheroid-qualified PHHs for different end points. These PHHs can also be used to set up co-culture liver spheroids with non-parenchymal liver cells such as Kupffer cells to model the in vivo responses to bacterial endotoxin (lipopolysaccharides or LPS) treatment, inflammatory responses of Kupffer cells in liver spheroids are demonstrated by IL6 and TNF-a secretion by ELISA assays. By comparing PHH only and co-culture liver spheroids, we show that both in intrinsic hepatotoxicity and immune-mediated cytotoxicity from Kupffer cells are underlining the mechanism of drug induced liver injury (DILI) by trovafloxacin. We extended this comparison to a selection of known DILI and control compounds including 5 tyrosine kinase inhibitors used in targeted cancer therapy. Both short-term 3D toxicity assay with a single dose and long-term 3D toxicity assay with repeated dosing regimen were used in this study for comparison. Our results demonstrate that using spheroid-qualified PHHs and spheroids made of different liver cell compositions can help the implementation and standardization of 3D liver spheroid model while providing flexibility for researchers to conduct both toxicity screening as well as investigative and mechanistic studies for liver safety assessment.

3D liver models (in vitro) are starting to be adopted by the pharmaceutical industry as an approach to de-risk drug induced liver injury (DILI). Recent approaches have focused primarily on the use of human hepatocytes (PHH) and comparing responses in 2D versus 3D cultures to a known reference list of DILI compounds, usually utilising a single measurement of cellular ATP. Here we have further developed this approach with the addition of multiparametric confocal high content imaging (HCI) to measure a range of endpoints including glutathione (GSH) content, mitochondrial membrane potential (MMP), reactive oxygen species (ROS) formation as well as cellular ATP content. We have evaluated two 3D models either PHH with the addition of Kupffer cells and the hepatic cell line, HepaRG in a 3D spheroid format. Characterisation of cytochrome P450 activity in liver microtissues (MTs) showed higher activities in HepaRG MTs compared to a single donor PHH MTs following 21 to 28 days in culture (CYP3A4: 210pM/min/MT HepaRG, 80pM/mn/MT PHH), indicating MTs are amenable to prolonged drug exposure. 57 DILI reference compounds (39 positive, 19 negative) were dosed through each MT for 14 days (with 4 repeat doses) and confocal HCI was determined in addition to cellular ATP content. PHH/PHH co-culture DILI was investigated in HepaRG MTs when compared to the overall response in the human hepatocyte MTs. However, donor specific sensitivity was observed across three different sources of PHH. In addition prediction of DILI was increased further when a multiparametric approach was taken when compared to measurement of ATP alone. Using HCI a sensitivity of 81.6% and a specificity of 100% was measured in PHH MTs whereas a sensitivity of 86.8% was observed in HepaRG spheroids and maintained the high specificity of 100%. 3D MTs consisting of either PHH or HepaRG cells gave an increased prediction of DILI over 2D approaches. In addition the prediction of DILI is also significantly improved by the addition of multi-parametric approaches when compared to only measuring cellular ATP. MTs of both types are amenable to high throughput screening, are cost effective and as such could play an important role in mitigating DILI risk in early drug discovery for the pharmaceutical industry.

Accumulating evidence implicates aryl hydrocarbon receptor (AhR) signaling in the regulation of liver fibrosis, which is a pathological condition characterised by excessive accumulation of extracellular matrix proteins. Liver fibrosis is mediated by myofibroblast precursors called hepatic stellate cells (HSCs). Upon chronic liver injury and inflammation, quiescent HSCs become activated and synthesise collagen type I. We previously reported that exposure to the high-affinity AhR ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) increases HSC activation in vitro, which raises the possibility that HSCs are directly targeted by TCDD. Recent reports indicate that chronic exposure of mice to TCDD elicits liver fibrosis with concomitant changes observed in parenchymal hepatocytes. The goal of this project was to determine how AhR signalling in HSCs and hepatocytes contributes to HSC activation during TCDD-induced liver fibrosis. Cre-Lox recombination was used to create mice with AhR-deficient hepatocytes (AhRfl/fl) or AhR-deficient HSCs (AhRfl/fl; Col1a1Cre). AhRfl/fl mice were used as controls. All mice expressed the AhR receptor. To induce liver fibrosis, female, adult mice were gavaged with TCDD (100 µg/kg) or peanut oil every four days for 92 days. In AhRfl/+/ mice but not AhRfl/fl mice, the TCDD-induced Col1a1 expression was similar to vehicle-treated mice. TCDD produced a similar effect in AhRfl/fl mice but had no effect on HSC activation in AhRfl/fl mice. Exposure to TCDD did not elicit robust hepatocyte necrosis, based on serum levels of alanine aminotransferase, but it did induce hepatic steatosis, and this effect was completely abolished in TCDD-treated AhRfl/fl mice. Finally, TCDD treatment increased inflammatory cell infiltration in the liver of all mice, regardless of AhR knockdown. We conclude that chronic TCDD exposure increases HSC activation indirectly through a mechanism that requires AhR signaling in hepatocytes. It is possible that hepatic steatosis contributes to HSC activation in TCDD-treated mice, whereas hepatocyte necrosis and hepatic inflammation do not appear to play a major role.
**1266 Elucidating Mechanisms of Intrahepatic Drug-Induced Liver Injury**


Drug-induced liver injury (DILI) continues to be a major cause of drug attrition and withdrawal from the market. While the development of bile-acid (BA) transporters (e.g., BSEP) has been implicated as a risk factor for DILI potential, not all potent BSEP inhibitors are associated with this risk. Given the complex and multifaceted nature of BA handling, other mechanisms that may be involved in intrahepatic cholestatic (IC) DILI progression such as mitochondrial injury secondary to BA accumulation in hepatocytes were investigated. To begin to study this, we assessed direct mitochondrial effects of 11 human relevant BA species using oxygen-based respiration studies in mitochondria isolated from rat liver. Similar to previous reports, we found specific hydrophobic BAs primarily inhibit mitochondrial ADP-stimulated respiration at physiologically relevant concentrations. Cholesterol oxidase activity, a suggestive for complex I inhibition, was noted in liver mitochondria from humans. We have further observed that liver mitochondria from humans have a higher sensitivity to mitochondria protein, while more hydrophilic BAs (e.g., C 4:0, GDCA, GCA, TCA) did not inhibit mitochondrial respiration appreciably (∆Ψ=500mV). However, a novel finding of this work identified the properties of unconjugated BA species (UDCA, CA, CDCA, DCA) to uncouple basal respiration versus their glycine/taurine conjugates contingent on complex I inhibition. Surprisingly, lithocholic acid (LCA) did not have any effect on mitochondrial respiration. We then assessed individual BA effects on cell-based respiration. While the trends were largely similar to those in isolated mitochondria, inhibition and not uncoupling was observed with LCA having the most prominent response. The disconnect between responses in the whole cell versus isolated system supports the complexity of primary and secondary mitochondrial toxicity effects that may ensue in an in vivo context. In conclusion, work presented here identifies a unique complex I specific mechanism for BA-mediated mitochondrial injury. These results add to the weight of evidence supporting drug-induced mitochondrial dysfunction coupled with increased accumulation of these BA species (mediated by inhibition of bile-acid transporters) increases risk for DILI. Other compensatory mechanisms and effects on nuclear transcription factors, downstream signaling, stress cell death/pathways are currently being investigated.

**1267 Effects of FGF15 on Expression of Genes Involved in Circadian Rhythm and Lipid Metabolism in Mouse Liver**

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Non-alcoholic fatty liver disease (NAFLD) is a growing epidemic that affects 30-40% of the general population. There is accumulating evidence suggesting that hepatic cirrhotic disruption is linked to NAFLD development. Fibroblasts growth factor 15 (FGF15), the mouse homologue of human FGF19, is an endocrine FGF secreted from the intestines in response to increased bile acid. It is critical in suppressing bile acid synthesis and improving insulin sensitivity. Direct effects of FGF15 on liver mitochondria have been previously shown to play an important role in stimulating the phases of priming and termination of liver regeneration. This study explored the mechanisms in prevention of NAFLD by FGF15. Total RNA was extracted from liver tissues collected from Wild-type (WT), FGF15 transgenic (TG), and FGF15 knockout (KO, loss-of-function) mice and relative mRNA expression levels were determined using quantitative RT-PCR. Results showed that mRNA expression levels of a major circadian gene, Per2, was significantly higher, whereas the expression levels of another major circadian gene, Ntr1d1, and a major circadian regulatory gene, Bmal1, were significantly lower in liver tissues from TG mice compared to those in KO and WT mice. Meanwhile, mRNA expression levels of other lipid metabolism genes, including Cyp4a, Lcn2, and Mtp, were significantly higher in TG vs. KO or WT mice. In addition, mRNA expression levels of inflammatory genes, Il6 and Tnf-a as well as Cd36, were also affected by FGF15, although none of them were statistically significant. Collectively, these results suggest that FGF15 modulates expression of genes involved in circadian rhythm and lipid metabolism, which might contribute to its potential preventive effect on NAFLD in mice.

**1268 Development of a High-Throughput iPSC-Derived Liver-on-a-Chip for Hepatotoxicity Detection**


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Hepatic injury accounts for two-thirds of drug development failures in the pharmaceutical industry. The current regulatory-accepted models for assessing hepatotoxicity include: i) rodent models, which are expensive, low-throughput, and overall have unreliable concordance with human hepatotoxicity; ii) standard two-dimensional (2D) in vitro systems, using liver cancer cell lines and primary human hepatocytes, which have only a marginally improved predictivity. This study sought to optimize a 3D in vitro model of the human liver and predictive hepatotoxicity assays by adopting the vascularized Liver Acinus MicroPhysiology System (vLAMPS) developed by the University of Pittsburgh Drug Discovery Institute into Mimetas’ high-throughput organ-on-a-chip platform. Extracellular matrix (ECM) gels and flowing medium can be patterned into the Organoplate®, allowing for solid tissue structures to form adjacent to continuously-perfused, tubular endothelial vessels. The resulting platform contains 96 x 3D microfluidic liver sinusoid mimics, including FUJIFILM Cellular Dynamics Inc. (FCDI) induced pluripotent stem cell-derived hepatocytes (iPSC hepatocytes; iCell® Hepatocytes 2.0) and stellate cells incorporated in an ECM gel, fed by nutrient perfusion from an adjacent endothelial and Kupffer cell-lined blood vessel. Viability of the co-culture remained stable (80-95%) for up to 21 days of culture. We report long-term maintenance of metabolic activity including CY3P4A, as well as albumin and urea production (1-21 days, both up to 20 µg/day/10^6 cells). Further, we report a significant drop in the viability of APAP-treated Organoplate® cultures while 2D cultures were unaffected by the same concentration of the hepatotoxicant, including acetaminophen (APAP). The cell viability assay revealed up to an 80% reduction in the viability of APAP-treated Organoplate® cultures while 2D cultures were unaffected by the same concentration of the hepatotoxicant. These studies display the feasibility of using our iPSC-derived 3D human liver model as a high-throughput screening platform for pharmaceutical and environmental hepatotoxicity.

**1269 AhR Signaling in Hepatocytes Is Required for Maximum Myofibroblast Activation by TCDD**

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The aryl hydrocarbon receptor (AhR) is a soluble, ligand-activated transcription factor that has been implicated in liver fibrosis. During fibrosis, chronic injury and inflammation drive the activation of myofibroblast precursors, namely hepatic stellate cells (HSCs), which produce collagen. We have previously shown that AhR activation by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) increases HSC activation in vitro and in a mouse model of liver fibrosis elicited by chronic carbon tetrachloride (CCL4) administration. It is possible that HSCs are direct, cellular targets for TCDD/AhR signaling. However, TCDD could also increase HSC activation indirectly through AhR-dependent events in hepatocytes that exacerbate liver damage and/or inflammation. The goal of this project was to determine the cell-specific consequences of TCDD/AhR signaling on HSC activation during liver fibrosis. Cre-Lox recombination was used to generate male mice in which the AhR was removed from either hepatocytes (AhR^fl/fl) or HSCs (AhR^fl/fl). AhR^fl/fl mice were used as controls. Mice (n=8) were treated with 1.0 ml/kg CCl4 every four days for 5 weeks, and TCDD (100 µg/kg) was administered during the final week of the experiment. HSC activation was measured by hepatic expression of αSMA and Colla1. Liver damage and inflammation were assessed based on serum alanine aminotransferase (ALT) levels and histological analysis, respectively. Results indicate that TCDD treatment increased HSC activation in the liver of all CCl4-treated mice, but not in wild-type mice. Treatment with TCDD marginally improved predictivity. This study sought to optimize a 3D in vitro model of the human liver and predictive hepatotoxicity assays by adapting the vascularized Liver Acinus MicroPhysiology System (vLAMPS) developed by the University of Pittsburgh Drug Discovery Institute into Mimetas’ high-throughput organ-on-a-chip platform. Extracellular matrix (ECM) gels and flowing medium can be patterned into the Organoplate®, allowing for solid tissue structures to form adjacent to continuously-perfused, tubular endothelial vessels. The resulting platform contains 96 x 3D microfluidic liver sinusoid mimics, including FUJIFILM Cellular Dynamics Inc. (FCDI) induced pluripotent stem cell-derived hepatocytes (iPSC hepatocytes; iCell® Hepatocytes 2.0) and stellate cells incorporated in an ECM gel, fed by nutrient perfusion from an adjacent endothelial and Kupffer cell-lined blood vessel. Viability of the co-culture remained stable (80-95%) for up to 21 days of culture. We report long-term maintenance of metabolic activity including CY3P4A, as well as albumin and urea production (1-21 days, both up to 20 µg/day/10^6 cells). Further, we report a significant drop in the viability of APAP-treated Organoplate® cultures while 2D cultures were unaffected by the same concentration of the hepatotoxicant, including acetaminophen (APAP). The cell viability assay revealed up to an 80% reduction in the viability of APAP-treated Organoplate® cultures while 2D cultures were unaffected by the same concentration of the hepatotoxicant. These studies display the feasibility of using our iPSC-derived 3D human liver model as a high-throughput screening platform for pharmaceutical and environmental hepatotoxicity.
1270 Does Triclosan Affect High-Fat Diet-Induced Non-Alcoholic Fatty Liver Disease (NAFLD) in Mice?

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Prior studies have suggested that the progression of NAFLD may be mitigated by the activation of constitutive androstane receptor (CAR) or peroxisome proliferator activated receptors (PPARs). The purpose of the current study was to investigate the effects of dual CAR and PPARα activator triclosan on preexisting NAFLD. Hepatic steatosis was established, as we previously reported, by administering a high-fat diet (HFD) ad libitum to male C57BL/6J mice for 16 weeks prior to chemical treatment. Controls were fed a low-fat diet (LFD). Mice were subsequently treated with vehicle or 100 mg/kg/day triclosan for another 2, 8, and 16 weeks by oral gavage. Dietary treatment continued throughout the whole study. PPARα and CAR, as measured by increased acyl-CoA oxidase (ACO) and peroxisome proliferator-activated receptor (PPAR) activity respectively, were activated by triclosan in the liver regardless of diets as expected. Expression of Cyp4a10, a target gene of PPARα, was induced concurrently by triclosan with a peak increase at 2 weeks exposure. Transcriptomic analyses showed that “PPAR signaling pathway” and “peroxisome” were significantly enriched after 8 weeks triclosan exposure. The mRNA level of CAR signature gene, Cyp2b10, was increased by triclosan treatment regardless of diet, with the highest fold-change seen at 16 weeks of exposure. The preexisting fatty liver seemed to have a protective role in triclosan induced CAR activation. Comparing to LFD-fed triclosan-treated mice, HFD-fed triclosan-treated mice had a significantly lower PROD activity across all time points studied and a trend of reduced expression of TNRFα and TNFβ versus TNRFα and TNFβ respectively. In conclusion, decreased HFD-induced elevation of serum ALT in mice, histological examination and quantification of hepatic triglycerides indicate no modulation of hepatic steatosis. Gene expression analysis showed that transcripts in both fatty acid uptake and oxidation pathways were upregulated, which may result in an overbalance of lipid metabolism. The expression of fibrosis markers such as Collα1 and Tinp1 also showed no significant change comparing triclosan-treated to vehicle controls in HFD-fed mice, although a decrease was observed. Taken together, these results demonstrate that triclosan treatment significantly activate both PPARα and CAR without impact on the hepatic steatosis.

1271 The Effect of Kupffer Cells on Hepatobiliary Transporters in Human and Rat Hepatocyte/ Kupffer Co-Culture Model


Non-parenchymal cells of the liver have a significant impact on hepatocyte activity, and it was also shown that they play in concert in the progression of hepatotoxicity. Kupffer cells (KCs), the resident macrophages of the liver are involved in response to many stresses of the liver, such as infection, toxins and ischemia. Activation of the KCs results in the secretion of stress factors, which in turn modulate the expression and function of transporters involved in the elimination of toxic endogenous compounds and xenobiotics. The aim of this study was to investigate the effect of KCs on the activity of transport proteins, which are essential in the maintenance of liver homeostasis and bile secretion. Freshly prepared primary human and rat hepatocytes were cultured in monolayers, or co-cultures with KCs in sandwich configuration. KCs were applied at a physiological (H/KC 3:1 - human; 2:1 - rat) or pathological (H/KC 3:1 - human; 2:1 - rat) ratio. All protocols were approved by the Institutional Animal Care and Use Committee and the Research Ethics Committee of the Medical Research Council in adherence to the declaration of Helsinki. Lipopolysaccharide (LPS) was used for KCs activation, and the effectivity of activation was confirmed by TNFα and IL-6 levels, which were measured by ELISA. Hepatocyte function was determined by monitoring albumin and urea secretion. The mRNA expression and transport activity of NTCP/Ntcp, BSEP/Bsep and Mrp2,3/Mrp2,3 were measured using their selective endogenous substrates taurocholic acid (TA) and bilirubin (B), respectively. LPS increased Bsep and MRP2,3/Mrp2,3 were measured using their selective endogenous substrates TAurocholic acid (TA) and bilirubin (B), respectively. LPS increased both activities in both human and rat cultures. Activated KCs decreased albumin secretion more effectively in rat than in human, though urea secretion did not change significantly. In rat cultures, both influx and efflux of TC increased in a KCs concentration dependent manner irrespective of KCs activation. In contrast, TC transport by human hepatocytes proved to be more sensitive. LPS treatment than to the amount of KCs. KCs decreased bilary transport of B in both human and rat cultures, which was accelerated by LPS. The sinusoidal efflux of B decreased just in rat co-cultures. In conclusion, the activity and expression of the transporters examined here were highly influenced by KCs. The direction and extent of alterations were markedly different in human compared to rat that calls the attention to species differences in case of liver injury. This study was supported by a GINOP-2.1.1.-15-2016-00826 grant.

1272 Biochemical Mechanisms of Orally Administered Phenobarbital in Minipig Liver


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Biochemical mechanisms of toxicity are often studied in rodents to establish a mode of action for adverse, chemical-induced effects. Phenobarbital (PB) has been shown to induce differential gene expression and CYP2B, CYP3A and UGT enzyme activities in rodent liver, resulting in increased thyroid hormone clearance and subsequent stimulation of cell proliferation in the thyroid leading to thyroid tumors after long-term exposures. This pathway has not been completely investigated in nonrodents. In this study, male Gottingen minipigs were orally administered 15 mg/kg/day PB for 6 days following by investigation of phase I and II liver enzymes (mRNA expression and enzyme activities), circulating thyroid hormone levels and differential gene expression in the liver in addition to standard toxicology evaluations. PB had no effect on body weight, hematology or clinical pathology. Treated minipigs had 42-46% increased liver weights with mild, diffuse hepatocellular hypertrophy versus the control group. PB reduced plasma concentrations of T3 (by 27% and 47%) and T4 (by 21% and 32%) vs respective controls. PB increased the expression of many phase I and II enzymes, with the most significant increase in CYP3A429 and CYP4A24, 5-fold increase in CYP1A2 and around 11-fold increase in CYP2B22 compared to the control group, with increases in mRNA expression of these genes. A 1.6-fold increase in T3-specific UGT phase I activity was observed in PB-treated animals, with a 1.6-fold increase in total UGT phase I activity. PB increased the expression of mucin 16 (MUC16) and decreased the expression of carboxylesterase 2 (CE2). Activation of the unfolded protein response (UPR) in a concentration-dependent manner was observed. The mRNA expression of these genes was upregulated in PB-treated animals, with a 1.6-fold increase in T3-specific UGT phase I activity and a 1.6-fold increase in total UGT phase I activity. PB increased the expression of mucin 16 (MUC16) and decreased the expression of carboxylesterase 2 (CE2). Activation of the unfolded protein response (UPR) in a concentration-dependent manner was observed. The mRNA expression of these genes was upregulated in PB-treated animals.

1273 A Comprehensive Landscape of the Temporal Dynamics of Cellular Stress Response Pathway Activation in Drug-Induced Liver Injury

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Drug-induced liver injury (DILI) is the most prevalent adversity occurring in drug development and clinical settings. Various liver toxicants cause cellular perturbations that lead to the activation of particular cellular adaptive stress response pathways. So far insight in the quantitative temporal responses of stress pathway activation remained fully unclear due to lack of adequate methods. In this study, we systematically determined the dynamics of key cellular stress response pathways in direct relation to the onset of cytotoxicity during drug exposure. We applied automated live cell high content confocal imaging of 13 different BAC-GFP HepG2 reporter cell lines representing various components of i) unfolded protein response (ATF4, XBP1, BIP and CHOP), ii) oxidative stress response (NRF2, SRXN1, HMox1), iii) DNA damage response (P53, P21, BTG2, MDM2), and iv) NF-κB signaling pathway (A20, ICAM1). For 14 different DILI compounds the concentration-dependent (from 1 until 100 Cmax) dynamics of stress response activation and cell death markers were followed for 72 h at 1-2 h time resolution. Quantitative image analysis captured the activation of stress responses at the single cell level and with distinct temporal dynamics. Interestingly, some compounds showed the partial activation of the unfolded protein response (UPR) in a concentration-dependent manner. At higher concentrations, the progressive UPR pathway ATF4/CHOP was found more prominently activated than the adaptive response XBP1/BIP. Compounds that activated specifically the ATF4/CHOP signaling branch affected significantly cell survival. Typically DILI compounds activated the oxidative stress response at early time points. On the other hand, the DNA damage response was only partially activated by some toxicants. The NF-κB signaling pathway is immediately activated after TNF-α co-exposure with the persistent expression of ICAM1 and A20. Altogether, our data suggest that the activation dynamics of stress response pathways is dependent on the DILI compound as well as predictive for the cell fate. In context of cellular adaptation and adverse cell fate, understanding the dynamics of cellular stress response pathway activation will provide an improved DILI hazard assessment at the early phase of pre-clinical drug development. This work was part of the Dutch-German ZonMW-BMBF SysBioToP project and the EU Innovative Medicine Initiative TransQST project. Grant agreement No. 116030.
**1274 In Vitro 3D Culture Systems for the Study of Human Liver Diseases**

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Presently, the most common culture system used to address in vitro human hepatic disorders and hepatobiliary functions is the 2D or 2D+ (collagen-matrix sandwich) monoculture systems of primary human hepatocytes (PHH). Although these models have proven to be very useful in the past, they reproduce neither the complete physiology of the human hepatocytes, nor the cellular complexity of the liver. With the aim of establishing in vitro culture models closer to the in vivo situation, we set up 3D mono- and multi-cellular spheroids, respectively composed of PHH, or PHH combined with non-parenchymal cells (NPCs). Here, we first described the process of spheroid generation and the viability of the formed spheroids. Then, we performed phenotypic and metabolic characterizations and found that 3D monocellular spheroids exhibit enhanced liver-specific functions, such as increased albumin secretion and higher metabolism of phase II substrates, compared to the same batches of PHH cultivated in 2D+. Furthermore, we performed whole transcriptome and microproteomic analysis in order to better characterize these models. Finally, we present preliminary results regarding their use as models for the study of i) human hepatic disorders, such as liver fibrosis, and ii) toxicological studies and drug testing applications, the latter targeting the hepatic stage of malaria, the deadliest parasitic liver infection. Thus, this present study provides a human microphysiological 3D system for in vitro liver disease modeling and toxicological studies as well as drug screening.

**1277 Human Liver-Chip Model for Drug Metabolism and Liver Safety Assessment**

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The pharmaceutical industry has an unmet need for predictive human models for drug metabolism and pharmacokinetics, drug-drug interactions, and drug-induced liver injury (DILI) that can better emulate human response to drugs. Current 2D liver models often fail to capture responses seen in the clinic, as the cellular microenvironment does not accurately reflect what is found in vivo. Here we applied a vascularized human Liver-Chip model that contains primary human hepatocytes, sinusoidal endothelial cells, and Kupffer cells, cultured under physiological fluid flow in a spatial configuration that recapitulates their cytoarchitecture in the liver. We demonstrated that albumin and urea are expressed and that CYP450 enzyme activity and protein expression were decreased. Induction profiles in human Liver-Chips showed improved functionality over conventional monolayer culture and were maintained in culture for >2 weeks. In the tri-culture model (with Kupffer cells) LPS stimulated secretion of TNFα in proportion to the Kupffer cell ratio, while tacrine stimulated the release of a number of cytokines in both the hepatocyte and endothelial cell channels. We tested benz bromarone, trazadone and two proprietary tool compounds, to evaluate the ability of a co-culture (hepatocytes and endothelial cells) Liver-Chip to assess DILI. The data demonstrated that the Liver-Chip provided a more sensitive response compared to conventional monolayer culture to toxicity. The hepatocyte/endothelium/human Kupffer cell co-culture system was developed to provide a proof of concept that HEPATOPAC® platform is amenable to hepatic stage of malaria, the deadliest parasitic liver infection. Thus, this present study provides a human microphysiological 3D system for in vitro liver disease modeling and toxicological studies as well as drug screening.

**1275 Multidrug Resistance-Associated Protein 4 (MRP4) Plays a Crucial Role in the Pathogenesis of Fatty Liver Disease**

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Hepatic steatosis without alcohol abuse (non-alcoholic fatty liver disease, NAFLD) is characterized by excess lipid accumulation in the liver that can impair various liver metabolic functions. Recently, our laboratory determined that genetic ablation of the efflux transporter Multi-drug resistance associated protein 4 (Mrp4) in mice increases hepatic lipid accumulation following partial hepatectomy, a model for hepatic tissue regeneration. The aim of this study was to further characterize this association between Mrp4 function and NAFLD development. To address this, we employed prolonged fasting, a model for studying hepatic lipid metabolism and NAFLD. In this study, both male and female wildtype (WT) and Mrp4 Knockout (KO) mice were either free-fed or fasted for 24 hr. Mice were then euthanized and liver, adipose tissue and plasma were collected. Lipid content in the form of triglycerides, free-fatty acids, glycerol, and cholesterol in plasma and liver samples was quantified. Hepatic lipid accumulation was also examined by Oil red-O staining of liver sections. Although no genotype- or gender-related changes in plasma lipids were detected, both male and female KO mice had greater hepatic lipid accumulation compared to WT mice. This was also confirmed by Oil red-O staining. Fed KO mice of both genders showed increases in hepatic triglycerides and free fatty acids, with no changes in cholesterol. By contrast, only fasted female KO mice had increases in hepatic free fatty acids levels, and not other lipids. Overall, this study supports the role of Mrp4 in hepatic lipid homeostasis and a genetic contributor to NAFLD and also document gender-related differences in hepatic lipid handling in the absence of Mrp4. Further studies are warranted to decipher this novel role of Mrp4 in hepatic lipid metabolism.

**1276 Demonstration of Hepatocyte-Targeted siRNA Transfection and Gene Silencing in the Micro-Patterned Hepatocyte Co-Culture System (HEPATOPAC)**

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The HEPATOPAC® model, an in vitro bioengineered co-culture of primary hepatocytes and fibroblasts, has demonstrated invaluable utility for liver-based safety, metabolism, and efficacy evaluation for small molecule drug candidates, due to its close resemblance to the in vivo liver. Here, we identify a method to specifically deliver small-interfering RNAs (siRNA) into the hepatocytes in the HEPATOPAC® co-cultures, by using a commercially available, non-liposomal transfection reagent that targets hepatocytes (PromoFectin-Hepatocyte). Upon the transfection of a fluorescent control siRNA, fluorescent signal was detected mainly in the hepatocyte islands, but not in the surrounding stromal cells. When siRNA targeting a cytochrome P450 enzyme was transfected in HEPATOPAC® cultures, a time-dependent reduction in the CYP activity following transfection was observed. The results provide a proof of concept that HEPATOPAC® platform is amenable to hepatocyte-specific siRNA transfection and siRNA-mediated gene knockdown, which can be useful in elucidating the hepatocellular mechanisms in various research areas, aiding in reaction phenotyping assessment, as well as in vitro safety and efficacy studies for novel RNA therapeutics.

**1278 Effects of Phenobarbital on Minipig Liver Gene Expression**

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Phenobarbital (PB) is a widely used anti-seizure drug. Oral administration of PB in rodents results in hepaticomegaly and induction of CYP450 xenobiotic metabolizing enzymes in the liver, and long term treatment results in hepatic tumors. While PB has been extensively studied in mice and rats, little is known about the impact of PB treatment on gene expression profiles in minipig liver. The present study was aimed at analyzing the effect of repeated acute oral administration of PB on minipig liver gene expression and comparing the data to liver enzyme induction and activity in a non-rodent model. For that purpose, three male Göttingen minipigs aged 4-5 months were treated orally with vehicle or 15 mg/kg PB for 6 days. On day 7, animals were euthanized and RNA was extracted from an 80 mg section of flushed liver. Affymetrix target preparation was performed using the Affymetrix 3’-IVT Plus kit and cRNA product was hybridized to Affymetrix GeneChip® Porcine Arrays. The differentially expressed genes were filtered using an absolute fold-change threshold at 1.5 with a corrected p-value lower than 0.05. Treatment with PB was well tolerated. A total of 161 and 139 probesets were found to be significantly down and upregulated in minipig liver, respectively, following PB administration. Induction of mRNA encoding for CYPs such as CYP2A19, CYP2B22 and CYP2C42, CYP3A9 and CYP3A46 was observed with mean fold changes varying between 31.19 and 1.51. Results were broadly concordant with those of relative quantitative RT-PCR and some of the corresponding enzymatic activities. Transcripts of phase II metabolizing enzymes such as UGTs, SULTs and GSTs were also found to be significantly upregulated by PB treatment in minipig liver. Pathways and ontology classification using the DAVID software revealed that the oxidoreductase, retinol metabolism, chemical carcinogene-
Lack of Multidrug Resistance-Associated Protein 4 (Mrp4) Does Not Alter Susceptibility towards Acetaminophen Toxicity

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Acetaminophen (APAP) is the most frequent cause of drug-induced liver injury in humans and a common chemical model to investigate genetic determinants of susceptibility to drug-induced liver injury (DILI). Previous studies performed in our laboratory identified the efflux transporter multidrug resistance-associated protein 4 (Mrp4) as an inducible gene in liver following toxic APAP exposure in both humans and rodents. In mice, prevention of hepatic Mrp4 induction following APAP administration increases susceptibility towards APAP hepatotoxicity. Collectively, these findings suggest that Mrp4 plays an important role in tolerance towards APAP-induced liver injury. To further study the role of Mrp4 in APAP-induced hepatotoxicity, we challenged 10-12 weeks old male wild type (WT, C57BL/6J) and Mrp4 knock out (Mrp4–/–) mice with either APAP (400 mg/kg in saline, ip), or vehicle. Following treatment, plasma and liver samples were collected at 12, 24 and 48 hr and liver injury was assessed by plasma alanine aminotransferase (ALT) activity and histopathological examination. No significant differences in plasma ALT levels or histological scores were observed between Mrp4–/– and WT mice, except at 12 hr where Mrp4–/– mice exhibited delayed death and hepatic necrosis compared to WT mice. Gene expression analysis indicates that lack of Mrp4 is associated with decreased expression of hepatic glutathione metabolism and drug metabolizing genes, such as glutamate-cysteine ligase modifier subunit (Gclm), Cyp3a11 and Mrp2 under basal conditions. These differences in gene expression were not observed in Mrp4–/– mice at any time point post APAP administration. In contrast to the gene expression data, Mrp4–/– mice had increased hepatic non-protein sulfhydryl (NPSH) content at 12 and 24 hr after APAP treatment. Although significant decreases in endpoints of liver injury were detected early after APAP treatment in Mrp4–/– mice, these changes were not sustained at later time points. In conclusion, our data indicate that lack of Mrp4 in mice does not alter susceptibility to APAP toxicity.

3D Spheroids from Nonhuman Primary Hepatocytes as an In Vitro Cell Culture Model


Currently used 2D sandwich cultures of primary hepatocytes is characterized by short longevity and lack of predictivity. Recent evidence suggests that putting primary hepatocytes in 3D hepatic spheroids offers major advantages in providing longevity and increased physiological relevance in modeling liver metabolism. Although a vast amount of data exist for human 3D spheroids, limited information is provided for animal 3D spheroids for their use in drug discovery and development. Here we show data that supports formation and use of 3D spheroids from rat (Srague-Dawley), mouse (CD-1), dog (beagle), and monkey (Cynomolgus) for drug testing. Rat (RTCP10), mouse (MSCP10), dog (DGP10) and monkey (MCP10) primary hepatocytes from Thermo Fisher Scientific were plated for spheroid qualified activity following the hepatic necrosis compared to WT mice. Gene expression analysis indicates that lack of Mrp4 is associated with decreased expression of hepatic glutathione metabolism and drug metabolizing genes, such as glutamate-cysteine ligase modifier subunit (Gclm), Cyp3a11 and Mrp2 under basal conditions. These differences in gene expression were not observed in Mrp4–/– mice at any time point post APAP administration. In contrast to the gene expression data, Mrp4–/– mice had increased hepatic non-protein sulfhydryl (NPSH) content at 12 and 24 hr after APAP treatment. Although significant decreases in endpoints of liver injury were detected early after APAP treatment in Mrp4–/– mice, these changes were not sustained at later time points. In conclusion, our data indicate that lack of Mrp4 in mice does not alter susceptibility to APAP toxicity.

CryoHepatoPearl: Ready-to-Use Cryopreserved 3D Human Liver Model


HepatoPearl is an innovative encapsulated 3D liver micro-tissue model developed by Cyprio using the CryoHepatoPearl technology. This technology was developed to overcome limitations of usual 3D culture models such as difficulties related to sphere handling and incompatibility to high-throughput screening. Viable and metabolically functional over 6 weeks, they have already been validated for drug-drug interaction studies and for the prediction of hepatotoxicity with 5 well-known compounds with different hepatotoxic potentials in acute (24 and 48h) and chronic (14 days, 3 dosing) exposures. For each compound, we observed a decrease in IC50 values with chronic conditions compared to those of acute exposure from 2 times for low concern compound (Acetaminophen) to more than 5 times for severe compound (Tolcapone). Moreover, after chronic exposure, IC50 values of low and chronically toxic compound far exceeded their non-cryopreserved counterpart. Tolcapone under it indicating its severe toxicity. Furthermore, by applying a margin of safety of 30 for the categorization of toxic potentials, we demonstrated that HepatoPearls efficiently categorized the same previously described toxic potential of these compounds. In order to avoid the problems linked to transport of fresh cells and facilitating the design of experiments for end users (in terms of timing and management), we thus developed a cryopreserved HepatoPearl model, named cryoHepatoPearls, as a ready-to-use tool for the previously mentioned assays. To do so, we directly froze the HepatoPearls after their encapsulation. After thawing, cells are still able to form spheroids in the same time range of their non-cryopreserved counterpart. Moreover, we did not observe a decrease in viability, metabolic activities and hepatic functionalities compared to non-cryopreserved HepatoPearls produced from the same batch between day 1 and 35 post thawing. We then measured cytochrome P450 basal enzymatic activity (CYP1A2, CYP2B6, and CYP3A4) and their mRNA expression inhibited by the concentration of albumin and urea secreted in the culture medium and observed that all these features are present in levels comparable to non-cryopreserved HepatoPearls. Hence, cryoHepatoPearls provide a unique storable liver 3D cellular model, metabolically and functionally equivalent to their fresh counterpart, offering an improved ease of experimental planning in pharmacokinetics, safety and toxicity studies.

Transient Vinyl Chloride Exposure Exacerbates High Fat Diet-Induced Hepatic Injury and Tumor Formation in Mice

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Vinyl chloride (VC), a common environmental pollutant, directly causes hepatic angiosarcoma and toxicant-associated steatohepatitis at high exposure levels. The risk for developing liver disease is thought to have been largely mitigated by lowering the OSHA exposure limits. However, we have recently shown that lower exposure levels (i.e.< OSHA exposure limits), which do not directly damage the liver, enhance injury caused by a high fat diet (HFD). Although these lower exposure levels are currently considered “safe”, the long-term impact of low-concentration VC in combination with a HFD is unknown. C57Bl/6J mice were fed HFD, or a low-fat control diet (LFD) for 1 year. During the first 12 weeks of feeding, mice were also exposed to VC using an inhalation chamber at concentrations below the current OSHA limit (<1 ppm) or room air for 6 hours/day, 5 days/week. The remaining 9 months of feeding, mice were not exposed to VC. Plasma and liver samples were collected for histology and determination of liver damage. As expected, chronic HFD feeding for 1 year caused significant hepatic injury, including steatohepatitis and fibrosis; 12 weeks exposure to VC at the beginning of HFD feeding exacerbated this normal tissue damage. Specifically, the increase in indices of fibrosis (Sirius red staining, TGF-beta levels, and propyl-4-hydroxylase levels) were significantly enhanced by VC exposure. Additionally, 1 year HFD feeding induced the formation of hepatic tumors in all mice, predominantly Ki67-positive adenomas, as well as damaged altered foci. The number and severity of these lesions was enhanced by VC exposure. The increase in indices of proliferation caused by HFD (e.g., Ki67 and cyclin D1) was enhanced by VC exposure. Importantly, 40% of the mice in this group also had an increase in CD31, and endothelium-derived tumors, which were characteristic of VC-induced hepatic angiosarcomas in humans. Taken together, these data suggest that although VC causes no overt hepatic injury in LFD-fed mice, it sensitizes the liver to other stressors (e.g., HFD) resulting in enhanced tumorigenesis. Importantly, these data raise concerns about potential for overlap between fatty diets (i.e. Western diet) and exposure to VC and the health implications of this co-exposure for humans. It also emphasizes that current safety restrictions may be insufficient to account for other factors that can influence hepatotoxicity.
Non-alcoholic fatty liver disease (NAFLD) is the most prevalent type of liver disease and currently affects ~30% of the population. With progression to non-alcoholic steatohepatitis (NASH), this disease can eventually lead to liver cirrhosis and failure. To date, there are no approved drugs for NASH treatment and drug development has been impeded by the lack of predictive in vitro models reflecting the complex pathology of NASH. Here, we present a human in vitro NASH model based on 3D microtissue technology. Engineered to incorporate the primary human hepatocytes, hepatic stellate cells, Kupffer cells (KCs) and liver endothelial cells (LEC), this model includes all the liver cell types that play a crucial role in disease initiation and progression. Upon treatment with free fatty acids and LPS in diabetic medium these microtissues showed key physiological aspects of NASH. Increased lipid accumulation within the hepatocytes could be detected as well as tissue secretion of pro-inflammatory markers, such as TNF-α, IL-6, IL-8, MCP-1, MIP-1α and αvβ5. Furthermore, lipotoxic stress stimuli increased expression of pro-fibrotic markers, such as collagen type I and a-smooth muscle actin (a-SMA), a marker of activated stellate cells. Further treatment with NAS stimuli resulted in increased deposition of ECM, indicating the activation of fibrotic pathways. In summary, we present a human 3D NASH model that recapitulates key biological aspects of the NAFLD spectrum of diseases, including inflammation, steatosis and fibrosis. Compatible with high-throughput screening approaches, this model is a powerful tool for assessing efficacy of anti-NASH drugs.

Iron-catalyzed formation of reactive oxygen species (ROS) increases after APAP overdose and triggers the mitochondrial permeability transition (MPT). Previous studies show that iron translocation from lysosomes into mitochondria by MCU promotes the MPT after APAP. Mfrn2 is also a pathway for mitochondrial iron transport. Here, our aim was to compare the roles of Mfrn2 and MCU in APAP hepatotoxicity. Hepatocytes isolated from Mfrn2 knockout (KO) and wildtype (WT) mice were treated with 10 mM APAP. Mitochondrial membrane potential and cell death were visualized by confocal imaging of rhodamine 123 (Rh123) and propidium iodide (PI), respectively. Mitochondrial chelatable Fe⁺⁺ was monitored by fluorescence quenching of mitochondrially targeted mitoferrofluor (MFF). For in vivo studies, Mfrn2 KO and MUC KO mice were treated with 300 mg/kg APAP. Liver injury was assessed by serum ALT and histology. Mitochondrial polarization and cell death were assessed by intravital multiphoton microscopy of Rh123 and PI. Progressive quenching of MFF began after ~6 h in both Mfrn2 KO and WT hepatocytes, signifying increased mitochondrial chelatable Fe⁺⁺. Mitochondria then depolarized after ~11 h followed by cell death. Of mice treated with APAP, ~85% of Mfrn2 KO hepatocytes were apoptotic. MFF quenching and mitochondrial depolarization in both WT and Mfrn2 KO hepatocytes. In vivo in WT mice, APAP increased ALT (5.104 U/L) and necrosis (23%) at 6 h, which increased further to 10,254 U/L and 53% in Mfrn2 KO mice. By contrast in MUC KO mice at 24 h, ALT and necrosis decreased to 465 units/L and 12%, respectively, compared to 6015U/L and 42% in WT. Intravital microscopy confirmed mitochondrial depolarization and necrosis in pericentral hepatocytes of WT mice, which was decreased in MUC KO mice. After APAP, MUC mediates uptake of chelatable Fe⁺⁺ into mitochondria, leading to ROS formation and mitochondrial dysfunction, whereas Mfrn2 may mediate Fe⁺⁺ release and protection.

Using 3D Human Liver Microtissues to Model NASH Progression In Vitro for Drug Discovery and Safety Testing


The compound 3-(3,5-dichlorophenyl)-2,4-thiazolidinedione (DCPT) is hepatotoxic in rats and exhibits cytotoxicity in a variety of cells, including the HepG2 cell line. DCPT toxicity in vitro may be due to its breakdown into different metabolites. In order to see how the presence of cells affects the breakdown of DCPT, the compound was incubated in HBSS with HepG2 cells at a concentration of 20,000 cells/ml and with MDCK cells. Results show that when incubated in HBSS, DCPT underwent hydrolysis into S-(3,5-dichlorophenyl)aminocarboxyl)thioglycolic acid (DCTA) after 30 min and continued to breakdown into 3,5-dichlorophenylisocyanate (DPI) within an hour. The concentration of DCPT also decreased steadily over time while concentrations of DCTA and DPI increased. When incubated with cells, DCPT broke down into DCTA after 30 min, but break down to DPI was not evident until the 24-hour time point. Additionally, the concentration of DCPT after 24 hours was much less than that of DCPT incubated in HBSS alone. This could indicate that cells accelerate the breakdown of DCPT and that the breakdown into DCTA is favored over DPI, explaining its low concentration. DCPT was also incubated with Madin-Darby Canine Kidney (MDCK) cells at a concentration of 20,000 cells/ml and in HBSS. In comparison to DCPT incubated in HBSS, the amount of DCPT in solution with MDCK cells decreased at all time points, while DCTA increased after 30 and 60 min, and DPI was present in small amounts after 60 min and 24 hours. These results may indicate that the cells are accelerating the breakdown of DCPT into DCTA and DCTA into other undefined compounds based on its significant drop in concentration after 24 hours. Comparatively, MDCK and HepG2 cells showed different results when it comes to the breakdown of DCPT. With MDCK cells, DCPT levels were slightly lower at all time points than that of the HepG2 cells; however, DCTA and DPI concentrations appeared to be very different. The concentration of DCTA after 30 and 60 min was much higher and decreased to much lower levels after 24 hours with MDCK cells. DPI was also present after 60 min of incubation with MDCK cells compared to 24 hours with HepG2. Overall, these results suggest that the breakdown of DCPT varies when it was incubated with different cell lines. The relevance of these results to cytotoxicity and potential cellular uptake of the compounds requires further investigation. This research was supported by the Department of Pharmaceutical Sciences.
1287 Transcriptomic Profiling of the Inter-Individual Variability of Chemical-Induced Cellular Stress Response Activation Using a Large Panel of Primary Human Hepatocyte Donors
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Drug-induced liver injury (DILI) remains a major concern for the clinic and pharmaceutical drug development. Given the diversity of DILI outcomes, it is crucial to improve the prediction of DILI liability for novel chemical entities at an early stage in drug development by integrating mechanistic understanding. Many DILI markers are known to influence the activation of stress response pathways in a cellular mechanism to overcome stress. Since some patients are more prone to develop DILI, it is key to understand what the inter-individual variability is in activation of these stress responses. To shed light on the variability of DILI susceptibilities among patients, we profiled the transcriptome of a panel of 50 cryo-preserved primary human hepatocytes (PHHs) derived from different individuals using TempO-seq technology with the expanded Tox21 S1500+ gene set. To evaluate stress response activation, cells were exposed for 8 or 24 h to a broad concentration range of tunicamycin for unfolded protein response, diethyl maleate for oxidative stress response, cisplatin for DNA damage response and TNFα for NF-kB signaling activation. Transcriptomic profiles were related to LDH leakage as a measure for cytotoxicity. The variance in the concentration-dependent stress response activation among individuals could be captured. Genes mostly reflecting the divergence in stress response activation were identified. For each stress response, hepatocytes were classified for sensitivity using maximum fold change across dose response and rank of departure from pathway-related gene sets. Correlation of sensitivity for stress response activation and their background such as disease status was identified. Next, the three most sensitive or insensitive hepatocytes for unfolded protein or oxidative stress response activation were exposed to various DILI compounds. Here, the variability in sensitivity for DILI compounds among hepatocytes and their respective stress response activation and cytotoxicity was captured. In conclusion, profiling of the inter-individual variance in chemical-induced stress response activation will aid in the improved understanding of the variance in susceptibility towards DILI among patients. Supported by EU-ToxRisk project (grant agreement No 681002) and IMI MIP-DILI project (grant agreement 115336).

1288 Improved Phenotypic Relevance of Primary Mouse Hepatocyte Spheroids Supports Development of an In Vitro Collaborative Cross Platform for the Evaluation of Genetic Susceptibility Factors Associated with DILI
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We are developing an in vitro Collaborative Cross platform to enable the rapid and cost-effective investigation of gene-by-treatment interactions associated with adverse drug response. The platform will contain primary cells isolated from the genetically diverse lines of the Collaborative Cross mouse population and cultured on multi-well plates to allow for multiple concentrations, treatment regimens, and endpoints to be assayed across replicate wells in a single experiment. Previously, we demonstrated the ability to culture primary mouse hepatocytes in 3D spheroids to support an initial focus on drug-induced liver injury (DILI). The objective of this study was to evaluate the phenotypic relevance of primary hepatocyte spheroids over time. Spheroids were generated from cryopreserved C57BL/6 mouse hepatocytes by spontaneous self-aggregation in ultra-low attachment 96-well plates. Mouse hepatocyte spheroids with well-defined parameters were observed within 5 days and morphology, ATP, and albumin levels were maintained for 2 weeks post spheroid formation. Enzyme activities for 4 major cytochromes P450 were measured on days 1, 7, and 14 post spheroid formation were comparable to that reported for primary human hepatocyte spheroids. Concentration-dependent decreases in viability were observed in response to acetaminophen treatment, and a leftward shift in the EC50 for viability was observed with increasing exposure time. Conifocal, high-content imaging demonstrated rotenone-induced effects on morphology, viability, mitochondrial function, and oxidative stress. Taken together, our results support improved phenotypic relevance of the spheroid model over time and the ability to multiplex mechanistic endpoints via cellular imaging. Furthermore, the use of 3D spheroids will decrease the number of cells required per well and as a result the number of cells (and animals) needed overall. We are now isolating and cryopreserving hepatocytes from Collaborative Cross lines and comparing C57BL/6 mouse hepatocyte spheroids to native liver and fresh hepatocytes using gene expression profiling and quantitative targeted absolute proteomics.

1290 Pharmacological Estrogens and Reduced Risk of Cognitive Deficits in the US Elderly Population
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Cognitive loss and brain health are growing public health concerns. Deficits in olfactory and cognitive functions are considered early indicators for neurodegenerative diseases such as Parkinson’s and Alzheimer’s. Epidemiological and clinical studies suggest estrogen use is associated with beneficial odds of cognitive impairment in later life, although findings have been mixed. Here, we evaluated cross-sectional estimate of cognitive and olfactory functions in past users of oral contraceptives (OC) and hormone replacement therapy (HRT) among the U.S. population 65 years of age and older using data from the National Health and Nutrition Examination Survey (NHANES). The surrogates of cognitive health in this study included the test scores based on the: 1) word learning and recall modules from the Consortium to Establish a Registry for Alzheimer’s disease (CERAD) (immediate recall and delayed recall); 2) the Animal Fluency test; and 3) the Digit Symbol Substitution test (DSST). For sensory functions, smell, phantom odor, and persistent taste in mouth over the past 12 months were included in the test scores. Chi-square test of independence was used to test the associations between the medical questions and the brain health indicators. Logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs). We found significant association of OR with 95% CI between the past OC and HRT and lowered risk of the cognitive deficits in adjusted models with suspected covariates (age, race, ethnicity, education, diabetes, alcohol use, high blood pressure, physical activity, smoking status) of cognitive functions. We did not observe a significant association for olfactory as well as taste functions as indicators of olfactory functional impairments in response to past use of OC and HRT. In summary, pharmacological use of estrogen is associated with decreased odds of cognitive impairment in the US elderly population. Prospective studies are needed to address the causal relationship between use of OC and/or HRT, and cognitive impairment, dementia and AD.
85-90% of PD cases are apparently sporadic in nature, consistent with an environmental impact on disease etiology. Epidemiological studies have further confirmed the role of environmental factors like pesticide exposure in PD development. Various genetic risk factors have been identified from genome-wide associated studies (GWAS), but available model systems have a limited ability to interrogate gene-environment crosstalk at scale. Here we propose using Drosophila as a model system to understand complex gene-environment interactions in PD. We have used our newly developed α-syn model in Drosophila, and the environmental toxicant rotenone to develop the model for studying these complex interactions. In this study, we have shown that flies expressing human α-syn specifically in neurons, showed age dependent motor deficits and neuronal loss. Further, seahorse bioassay on Drosophila brain revealed that human α-syn expression induced mitochondrial dysfunction in these flies. Further, RNA-sequencing and proteomics analysis revealed that some key lipid metabolism pathways are altered in this model system. Previous studies have shown that mitochondrial complex I inhibition alters lipid metabolism in neuronal cells. Interestingly, our study showed that exposing these flies to the environmental pesticide, rotenone, a complex I inhibitor, exaggerated behavioral deficits and reduced lifespan in human α-syn expressing flies. Moreover, α-syn induced mitochondrial dysfunction also worsened in flies exposed to rotenone. These studies indicate that an environmental factor can be a modifier of a genetic factor in PD and we can use Drosophila to investigate these complex gene-environment interactions. To further validate our model, we selected four key genes which have been implicated by GWAS in PD. Next, we systemically knocked these genes down in neurons and glia separately and exposed the flies to pesticide to identify the mechanisms of pesticide toxicity by performing behavior assays and measuring dopamine level. In summary, we have developed a model to investigate genetic modifiers of pesticide toxicity, which may provide opportunities for the identification of personalized drug targets. Forward genetic screening using this model system, coupled with multiplexing technology can identify key pathways and drug targets.

**Development of Gene-Environment Interaction Model in Drosophila for Neurodegenerative Disease: A Step towards Personalized Medicine**

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Alzheimer’s disease (AD) is a neurodegenerative disorder associated with aging and characterized by decline in cognitive function. Currently, there is no effective treatment in slowing the progression of AD. However, several studies have shown that natural compounds found in fruits and vegetables can improve memory impairment. Indole-3-carbinol (I3C) is a phytocompound present in edible cruciferous vegetables. It has been reported that I3C produces 3,3'-diindolymethane (DIM) in the acidic environment of the stomach. This study was designed to investigate the effects I3C and DIM on Aluminum Chloride (AlCl₃)-induced AD in male Wistar rats. Forty-two rats were divided into 6 groups of 7 animals each. Animals in groups 1, 2, 3 and 4 received corn oil (2mg/kg), I3C (10mg/kg), DIM (10mg/kg) and AlCl₃ (100mg/kg) for 4 weeks prior to AlCl₃ (100mg/kg) challenge for 8 weeks. Brain silver staining observations, immunohistochemistry assays (Amyloid beta 1-42 (Ab), amyloid precursor protein (APP), B-cell lymphoma 2 (Bcl-2), Bcl-2-associated X protein (BAX) and immunofluorescence assessment of A Disintegrin and metalloproteinase domain-containing protein 10 (ADAM 10) of rat brains revealed that AlCl₃ intoxication decreased α-secretase expression with elevated expression of insoluble plaque and apoptosis. However, pre-treatment with I3C and DIM prior to AlCl₃ exposure significantly reverted the ADAM 10 expression, amyloid protein and apoptosis markers in the studied regions. These results, however, require an animal model treated with I3C and DIM prior to AlCl₃ exposure. The results obtained from the study showed that I3C and DIM reversed memory loss caused by aluminum chloride in rats by attenuating synaptic impairment, apoptosis, and amyloidogenic pathways.

**Long-Term Swimming Exercise in Caenorhabditis elegans Improves Mitochondrial Health and Protects Animals from Age- and Toxicant-Induced Degeneration**

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Age-related chronic diseases, including neurological disease and cancer, are well-recognized to involve contributions from genetic and environmental factors. However, the role of lifestyle in the initiation and progression of chronic disease, and how it interacts with genetic and environmental factors, is far less studied. Exes of providing noted benefits in humans, including reducing risk of age-associated chronic diseases. However, molecular mechanisms underlying these protections and how those mechanisms may impact exposure risk from environmental chemicals have remained elusive, partly due to the high cost and time investment of long-term exercise intervention studies in mammals. We have developed a long-term exercise intervention in the nematode C. elegans by subjecting them to twice daily swimming during their entire reproductive adulthood (6 days). Following exercise, we assessed mitochondrial health and general physiological health in aging animals. We also exposed the animals to common mitochondrial toxicants (rotenone and arsenic). Exercise protected from age-related decline in mitochondrial morphology (p<0.05) and spare mitochondrial respiratory capacity (30% increase from exercise, p<0.05). We observed modestly increased lifespan in exercised animals (median lifespan 19 days) compared to non-exercised animals (16 days; p<0.05). Exercised animals were markedly protected against oxidative stress with increased lifespan (19 days; p<0.05) and spare mitochondrial respiratory capacity (30% in exercised animals vs. 20% in non-exercised animals). In summary, swimming exercise provides effective intervention in C. elegans against age-related mitochondrial and physiological decline and toxicant exposure. This novel long-term exercise model will allow toxicologists to take advantage of the short lifespan and genetic power of C. elegans to dissect the mechanisms that mediate exercise modulation of exposure risk.

**Induction of Autophagy in Mouse Neuroblastoma Cells by Rabies Lyssavirus Infection**

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Autophagy (ATG) is induced by the condition of extra and intracellular stress stimuli or nutrient starvation. Interestingly, ATG has the immunological function such as multiple innate and adaptive immune pathways involved in the control of inflammation, removing endogenous inflammasome agonists through effects on the secretion of immune mediators. Rabies lyssavirus (RABV) is highly neurotropic and induces the different degree of ATG during the infection according to their viral pathogenicity. In this study, the kinetics of ATG focused on the accumulation of autophagosome during the infection of RABV HEP Flury (HEP) strain to mouse neuroblastoma cells (MNA). MNA cells were propagated and used ATG induction. HEP strain propagated in MNA cells was determined its infectious titer by a focus forming unit (FFU). MNA cells were exposed to 20 FM rapamycin well as a positive control of ATG. After the day 0, 4, 6 and 24th treatment with lysis buffer, MNA cells were examined by the western blotting (WB). LC3-II signal compared to LC3-I was higher in MNA cells treated with rapamycin and infected with HEP strain than MNA cells without any treatment. LC3-II /LC3-I signal was decreased in MNA cells treated with rapamycin after 4 h, however LC3-II /LC3-I signal in MNA cells infected HEP strain was not decreased. The highest LC3-II /LC3-I signal in MNA cells treated with rapamycin was shown at 6 h and it was decreased at 24 h. On the other hand, LC3-II /LC3-I signal in MNA cells infected with HEP strain was not decreased after 6 h and kept high LC3-II /LC3-I signal after infection. RABV N protein of HEP strain was increased visibly at 24 h. As levels of endogenous LC3-II in MNA cells were correlated with an increased number of autophagosomes, the signal of ATG induction was induced by the infection of RABV HEP strain induced ATG. This suggests that the infection of HEP strain pulled the trigger of ATG signal induction during early infection and continued the induction of ATG signal. It was reported that RABV was inactivated through the suppression of ATG by mTOR-dependent autophagy signaling pathway. But kinetics and mechanism of causal interrelation between RABV and ATG was not yet proved to satisfactory extent in RABV infected cell. Therefore, we are studying further analysis immunologically and histopathologically to understand the kinetics of ATG on the accumulation of autophagosome during the infection of RABV HEP strain to mouse MNA cells.
Evaluation of Role of Pericytes in Cerebral Amyloid Angiopathy (CAA) Model of Alzheimer’s Disease (AD)


Excessive deposition of amyloid-beta (AB) plaques and neurofibrillary tangles are two salient features of AD. Among most AD patients, 75-80% of them show CAA. CAA occurs because of deposition of plaques in the microvessels, a key component of neurovascular units (NVU). These endothelial cells are surrounded by pericytes, another component of NVU. Pericytes regulate blood flow in the brain and provide support to the microvessels and eliminate toxic molecules from the brain by an efflux mechanism. In this study, we evaluated the role of pericytes in a mouse model of CAA which shows AB deposition in the vessels in addition to parenchyma. To visualize pericytes in the brain, Fluoro-Ruby (FR)-dextran, a retrograde tracer was injected bilaterally into lateral ventricles of mouse brains. In addition, CD31 immunolabeling was performed to evaluate the integrity of the endothelial cells of the brain. Fluoro Jade C labeling was also done to evaluate neurodegeneration in the brain. FR labeled pericytes were dystrophic in nature in the CAA mice compared to their wild type counterparts. The results also showed that compared to the wild type, there was a higher number of pericytes in the cortex but fewer pericytes in the hippocampus of the CAA mice. Most FJC labeled axonal degeneration was prevalent in the hippocampus with a moderate amount in the cortex. While the number of endothelial cells were increased in the CAA mice compared to wild type mice; the area fraction and intensity of the endothelial cells in the brain of the CAA mice were significantly less than that found in the wild type. These findings suggest degeneration of the pericytes and endothelial cells and ischemia of the vessels in the cortex and hippocampus resulting from deposition of AB plaque deposition in the microvasculature of the brain and CAA could also cause axonal degeneration.

Decreased NRF1 Activity Contributes to Neurogenesis Deficits in Alzheimer’s Disease (AD)

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AD is a heterogeneous, multifactorial disease, and a major challenge in AD research is to fully understand the multiple etiologies and age-related pro-dromal processes involved. Hippocampal atrophy and cognitive deficits in AD patients are associated with marked alterations in neurogenesis. Deposits of AD-toxins, amyloid-β42 (Aβ42) and p-tau, lead to decreased neurogenesis and synaptogenesis contributing to memory deficits and learning impediments. There is an urgent need for a direct and targeted approach to identify molecular factors associated with neuronal loss and neurogenesis deficits in AD. Adult hippocampal traits-proliferation, survival/maintenance of new-born neurons and astrocytes are controlled by a wide range of gene expression. To date, over 250 genes have been implicated in adult hippocampal neurogenesis although causal NRF1 signature genes involved remain unknown. The primary objective of this study is to identify the key NRF1 transcriptional regulatory modules controlling neurogenesis and neurodegeneration in AD patients. Since adult neurogenesis is polygenic, it likely depends on multi-target gene interactions of NRF1 regulating gene expression, protein abundance, epigenetic state, and signaling activity. We hypothesize that NRF1 gene networks specific to neuronal and astrocytic become dysregulated during AD pathogenesis. Through transcriptomic data from four independent AD studies, we found decreased NRF1 activity in AD brain compared to normal controls. This also allowed us to identify a core set of NRF1 target genes down-regulated in AD. We characterized key molecular pathways associated with these genes including ALAS1, TRIM37, WRB, POP7 and STXBP1. Using Comparative Toxigenomics Database, we found 255 unique chemical substances interacting with at least one of these genes. We then employed Bayesian machine learning to determine gene relationships in various brain regions of AD patients compared to non-AD individuals stratified by age and gender. NRF1 regulated genes that show significantly decreased expression in AD brain tissue are also regulated by estrogen and endocrine disrupting compound bisphenol A (BPA), and have distinct gene networks in brain tissue from Alzheimer’s patients. NRF1 target genes, SOX2, SOX2-OT, NR2E1, MAPT and PSEN2, are associated with Alzheimer’s. Clinical confirmation of our findings will have significant impact on our understanding of NRF1 as a valuable biomarker for AD diagnosis and prognosis and will provide strong rationale for future studies to develop NRF1 signaling based therapeutics for Alzheimer’s disease.
individuals with ND, including Alzheimer’s Disease, Parkinson’s Disease and Amyotrophic Lateral Sclerosis, similar to those reported by us in neurotoxicity studies. Autoantigens (AAg) have included neurofilaments (NF), and glial proteins (glial fibrillary acidic protein (GFAP) and myelin basic protein (MBP)), among others. To investigate the prevalence and titers of NAB against a panel of these AAg in horticulturists exposed to pesticides, 106 (45-6 years old; 73 male; 33 female) active farmers residing in the Nile Delta with varying duration (5-40 years) of agricultural activity were recruited. According to records, all participants had occupationally used multiple classes of pesticides including organophosphates, carbamates, dipyr idimido and aminophosphonates. IgG titers against axonal NF-H, GFAP and MBP were significantly more prevalent compared to IgM titers against the same antigens (p<0.0001-0.0001), in contrast to NF-L and NF-M, where IgM was more prevalent (p<0.05). Furthermore, NAB IgG titers against NF-H, GFAP and MBP were significantly higher (p<0.00001-0.0001) compared to IgM against the same AAg. For NF-L and NF-M, IgM was the higher isotype (p<0.003-0.007). Stratification by NAB score (total IgG detected of 10 measured) indicated that the high NAB group had increased titers against NF-L and NF-M, and IgM titers against NF-H, GFAP and MBP significantly differed from those with higher scores (p<0.0001-0.04). Since IgG is the class associated with development of immunological memory and acute insult, its greater prevalence against NF-H and glial proteins suggests a longer term axonal and glial involvement, and a more chronic involvement of NF-L and NF-M, as suggested by IgM prevalence. This preliminary study indicates that exposure to pesticides with known neurotoxic potential results in detectable levels of serum NAB similar to those reported in other neurotoxic exposures and ND. A follow-up and expanded study incorporating neurological exam and genomic profile is underway.

**1300 Diesel Exhaust Dysregulates Markers of Disease-Associated Microglia**

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An increasing amount of evidence indicates an association of traffic-related air pollution, such as diesel exhaust (DE), with Alzheimer’s disease (AD). At present, how inhaled environmental pollutants impact central nervous system (CNS) and neurodegenerative diseases remains poorly understood. In recent decades, the importance of microglia, the parenchymal myeloid cells of the CNS, to the neurodegenerative process has become increasingly clear. Currently, little is known about the mechanisms through which DE may impact the microglial phenotype associated with containing AD plaques (Disease-Associated Microglia, DAM). TREM2 (Triggering Receptor Expressed on Myeloid Cells 2) is a myeloid-cell specific membrane protein and its increased expression is necessary for microglia to obtain the DAM phenotype. Further, inactivating TREM2 mutations are associated with increased AD risk. At present, how DE might affect TREM2 is unknown. To begin to address these issues, twelve- to fourteen-week-old male Wistar Kyoto rats were exposed to DE (0, 50, 150, 500 µg/m³) by inhalation for 4 weeks (4 hours/day, 5 days/week). Frontal cortex mRNA expression was analyzed by qPCR to discern the effects of DE on pro-inflammatory and DAM markers. Tn1C expression demonstrated a step-wise increase relative to dose. However, the DAM markers CX3C chemokine receptor 1 (CX3CR1), TREM2 and lipopolysaccharide lipase (LPL) exhibited an inverted-U pattern of expression, where an increase in expression at 50 and 150 µg/m³ was observed, but the highest concentration of DE decreased expression suggesting that higher levels of DE exposure may impair the DAM response. Analysis of frontal lobe TREM2 protein by ELISA revealed that full length TREM2 protein levels were diminished at all concentrations of DE, while soluble TREM2 (sTREM2) protein demonstrated a dose-dependent decrease with DE concentration. Together, these data support that DE perturbs the DAM phenotype and in particular, decreases TREM2 protein expression. Given the role of the loss of TREM2 function in elevated AD risk and an impaired DAM phenotype, these findings provide much needed insight into the potential underlying mechanisms of how air pollution affects AD.

**1301 The Selective Group I Metabotropic Glutamate Receptor Agonist 2-Chloro-5-Hydroxyphenylglycine (CHPG) Enhances BDNF and Reverses Demyelination**

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The demyelinating agent cuprizone (0.2%) causes a decrease in brain-derived neurotrophic factor (BDNF) and myelin proteins with an upregulation of metabotropic glutamate receptors (mGluRs) in astrocytes within the lesion site following 4-6 weeks of treatment. Similarly, patients with multiple sclerosis not only have deficits in myelin, 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 application of 2-chloro-5-hydroxyphenylglycine (CHPG), by using the clinically relevant approach of a peripheral injection. Cuprizone or identically processed control feed was fed to adult mice for 4 or 6 weeks prior to intraperitoneal (ip) injections of saline vehicle or CHPG (20 or 40 mg/kg). CHPG increased levels of BDNF and myelin proteins 24 hours after injection and this effect lasted up to 3 days. Myelin proteins increased without increases in CC1+ mature oligodendrocytes at 24 hours, suggesting that CHPG increases myelin protein per cell. Interestingly, CHPG did not alter BDNF, myelin protein numbers or neurons of CC1+ oligodendrocytes in control-fed mice. Furthermore, BDNF and myelin proteins remain elevated following administration of CHPG every other day for 2 weeks, suggesting that CHPG’s effects can be maintained over time. The same dosing regimen of CHPG also increases myelin thickness and the number of myelinated fibers following cuprizone as analyzed by transmission electron microscopy. To begin to elucidate the receptor responsible for CHPG’s actions and the site of action, the selective mGluR-5 antagonist 2-methyl-6-(phenylethynyl) pyridine (MPEP) was injected directly into the lesion site prior to ip injection of CHPG. While CHPG treatment increased BDNF and myelin proteins in the absence of MPEP, and aggregation of the protein alpha-synuclein leading before CHPG, suggesting both a role of mGluR-5 in mediating the actions of CHPG and that CHPG injected peripherally acts within the lesion site. Taken together, these data suggest that selectase mGluR Group 1 agonists such as CHPG may be a therapeutic approach for treating demyelinating diseases by increasing the levels of BDNF and improving myelination, possibly through astrocytic mGluR-5. Support: NIH NS036647, T32ES007148; F31NS098642 and NIOSH RG 4257BA1.

**1302 Using the Transgenic Zebrafish Model to Compare Mechanisms of Action of Two Environmental Toxics with Opposite Correlations to Parkinson’s Disease Development, DEPe and CSE**


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 loss of dopaminergic neurons in the substantia nigra, neuroinflammation, and aggregation of the protein alpha-synuclein leading to the formation of Lewy bodies. Epidemiological studies have reported a positive association between exposure to traffic-related air pollution (AP) and development of PD, but an inverse association between cigarette smoking and development of PD. We used zebras as a model organism to uncover the molecular mechanisms of action that are responsible for these opposing effects. Transgenic zebrafish embryos were treated with diesel exhaust particulate extract (DEPe) or cigarette smoke extract (CSE) to determine the effects of exposure to these toxicants on neuron health, neuroinflammation, and behavior. DEPe exposure led to a significant loss of aminegic neurons in the ZF brain and an activated microglia. CSE exposure led to a significant loss of aminegic neurons in the ZF brain and no activation of microglia. There was also significantly decreased light-cycling behavior in both DEPe and CSE treated embryos. The opposite effects of DEPe and CSE exposure on the activation of microglia may shed light on molecular mechanisms responsible for the anti-relationship of the two exposures with development of PD. Ultimately, these studies will help elucidate the mechanisms by which AP contributes to neurodegeneration in PD, and conversely, the mechanisms by which cigarette smoking is linked to lowered risk of PD development.

**1303 Pharmacological Activation of Prokineticin-2 Signaling Protects against Dopaminergic Neurodegeneration in MPTP and Mitopark Rodent Models of Parkinson’s Disease**

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Nigral dopaminergic neurons are highly vulnerable to neurotoxic stress but key signaling mechanisms underlying this enhanced vulnerability is not well understood. We recently reported that Prokineticin-2 (PK2), a recently discovered mammalian homolog of mamba snake venom, is highly induced in Nigral dopaminergic neurons are highly vulnerable to neurotoxic stress but key signaling mechanisms underlying this enhanced vulnerability is not well understood. We recently reported that Prokineticin-2 (PK2), a recently discovered mammalian homolog of mamba snake venom, is highly induced in Parkinson’s Disease and is caused by both genetic and environmental factors. The main hallmarks of PD include loss of dopaminergic neurons in the substantia nigra, neuroinflammation, and aggregation of the protein alpha-synuclein leading to the formation of Lewy bodies. Epidemiological studies have reported a positive association between exposure to traffic-related air pollution (AP) and development of PD, but an inverse association between cigarette smoking and development of PD. We used zebras as a model organism to uncover the molecular mechanisms of action that are responsible for these opposing effects. Transgenic zebrafish embryos were treated with diesel exhaust particulate extract (DEPe) or cigarette smoke extract (CSE) to determine the effects of exposure to these toxicants on neuron health, neuroinflammation, and behavior. DEPe exposure led to a significant loss of aminegic neurons in the ZF brain and an activated microglia. CSE exposure led to a significant loss of aminegic neurons in the ZF brain and no activation of microglia. There was also significantly decreased light-cycling behavior in both DEPe and CSE treated embryos. The opposite effects of DEPe and CSE exposure on the activation of microglia may shed light on molecular mechanisms responsible for the anti-relationship of the two exposures with development of PD. Ultimately, these studies will help elucidate the mechanisms by which AP contributes to neurodegeneration in PD, and conversely, the mechanisms by which cigarette smoking is linked to lowered risk of PD development.
plays a major compensatory protective function during neuronal injury. While we showed that AAV-mediated PK2 overexpression protects against MPTP-induced neurodegeneration, CNS delivery of PK2 via recombinant protein or gene transfer remains challenging. In this study, to harness the protective potential of PK2 activation, we utilized a small-molecule PK2 receptor agonist, iS2O, to pharmacologically activate PK2 signaling pathway. iS2O induced intracellular Ca\(^{2+}\) in CHO cells overexpressing PK2 receptor, suggesting that iS2O can pharmacodynamically engage the receptor PKR1. PK2 activation by iS2O also conferred significant protection against MPP\(^{+}\) neurotoxicity in N27 dopaminergic cells. Importantly, we assessed the neuroprotective property of iS2O in a subacute MPTP mouse model of PD, wherein it restored levels of dopamine, and preserved dopaminergic neuron bodies and neuron fibers in the substantia nigra of MPTP-treated mice. Next, to reduce systemic exposure to iS2O, we intranasally administered iS2O in MitoPark mouse model, a chronic, genetic model of PD that harbors selective TFAM loss-of-function mutations in DAergic neurons, leading to manifestation of Parkinsonian symptoms by age 12 wk. iS2O administration significantly improved the locomotor activity and attenuated severe nigrostriatal degeneration in MitoPark mice. Additional studies revealed that iS2O treatment decreased fluorescent apoptotic cell staining in the substantia nigra (SN), and neuroinflammatory IBA-1 expression in SN, striatum of MitoPark mice. Pharmacokinetics studies via LC/MS analysis indicated that intranasally-treated iS2O becomes rapidly bioavailable in the brain. Together, our results indicate that pharmacological modulation of PK2 signaling by the receptor agonists could be an efficient and viable option to harness the neuroprotective effects of PK2 signaling against PD neurodegeneration. R01 NS078247, R01 NS088206.

1304 Manganese Exposure Induces the Release of Exosomes Containing Misfolded α-Synuclein by Impairing Endosomal Trafficking and Protein Degradation Machinery

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Environmental exposure to excessive manganese (Mn) increases the risk of chronic neurological diseases, including Parkinson’s disease (PD). Aggregated α-synuclein (aSyn) is a key pathophysiological characteristic of PD. Oligomeric proteins like aSyn can be released from neurons by exosomes, facilitating the spread of misfolded proteins to neighboring cells, which can trigger a neurotoxic response. We recently discovered that Mn enhances the release of misfolded aSyn via exosomes from dopaminergic neurons, but the underlyung molecular mechanisms are unclear. To better understand the Mn-induced release of exosomal aSyn, we examined how Mn modulates endosomal protein trafficking and misfolded protein degradation. We exposed MN9D dopaminergic neuronal cells stably expressing human wild-type (WT) aSyn (MN9D-aSyn) to Mn (300 μM) for 24 h. Mn significantly suppressed expression of the key endosomal recycling protein Rab11a, both at the protein and mRNA levels, suggesting Mn downregulates endosomal recycling mechanisms, thus forcing late endosomes to mature into multivesicular bodies (MVBs). Moreover, ectopic expression of WT Rab11a significantly mitigated exosome release from iS2O-treated and Mn-exposed MN9D-aSyn cells. Aggregated aSyn expression of mutant Rab11a (S25N) increased exosome release. Intriguingly, 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 the plasma membrane, suggesting Rab27a upregulation contributes to Mn-induced exosome release. Since aggregated aSyn can get degraded via the autophagic/lysosomal degradation pathway, we examined if Mn impairs this pathway to promote exosomal aSyn release. Our Western blot analysis of MN9D-aSyn cells shows Mn upregulated the expression of the autophagosomal markers LC3-II and Beclin-1, but downregulated the lysosomal marker LAMP2, suggesting an impairment of autophagosome formation following Mn exposure. Results from other key lysosome function assays, such as LysoTracker staining and Cathepsin D activity assay, confirmed Mn-induced lysosomal dysfunction. Taken together, these novel findings demonstrate that Mn compromises endosomal trafficking and lysosomal function, thereby promoting the exosomal release of misfolded aSyn. NIH grants ES026892, NS088206 and the Eugene & Linda Lloyd Endowed Chair.

1305 Development of an Electrochemiluminescence-Based Assay to Characterize Pyroptosis-Related Proteins in Plasma Obtained from Parkinson’s Disease Patients

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Parkinson’s disease (PD) is a highly prevalent neurodegenerative disease affecting approximately 5 million people worldwide. Neuroinflammation is a widely recognized aspect of PD; however, the impact of inflammation on PD incidence and progression remains unclear. Exposure to environmental toxicants, including pesticides, heavy metals, and industrial solvents, has been implicated in PD risk, but the molecular basis by which toxicants impact PD progression is not completely characterized. Inflammases are pro-inflammatory intracellular protein complexes containing pattern recognition receptors capable of responding to sterile triggers by initiating inflammation and a subcategory of programmed cell death called pyroptosis. Our lab has previously shown that loss of NLPR3, the pattern recognition component of the NLRP3 inflammasome, in mice mitigates the development of PD symptoms resulting from exposure to the pesticide and mitochondrial toxin rotenone. More recently we observed expression of NLPR3 in the mesencephalon in late-stage PD patients. Based on the recognition that inflammasomes are activated in PD and cytosolic proteins are released during the process of pyroptosis, our lab has developed electrochemiluminescence-based immunoassays for the detection of pyroptotic proteins including NLR family pyrin domain containing 3 (NLPR3) and Gassdermin D (GSDMD). Utilizing this method, we have compared NLPR3 and GSDMD protein levels in human plasma samples collected from PD patients and age-matched controls. The detection of the downstream NLPR3 inflammasome targets, including GSDMD and IL-18, suggest that we may be able to monitor the activity of inflammasomes and evaluate pyroptotic processes in plasma. To complement these biochemical studies we have collected surveys from PD patients and controls detailing potentially inflammatory lifestyle factors, occupational exposures, and medical history information to identify environmental risk factors associated with circulating inflammasome and pyroptosis-associated proteins. Our study will allow us to correlate levels of inflammasome and pyroptotic activity with environmental exposure data to elucidate their relationship to PD diagnosis.

1306 Zebrafish Transgenic Model for Studying the Dynamics of Mitochondrial Dysfunction Induced Epigenetic Hyperacetylation: Relevance to Environmentally Linked Parkinson’s Disease


Growing evidence implicates occupational exposure to pesticides as an important risk factor for the development of Parkinson’s disease (PD). Exposure to neurotoxic pesticides induces mitochondrial dysfunction and oxidative stress, both of which are key pathological hallmarks of PD. We recently demonstrated that exposure of dopaminergic neuronal cultures to the neurotoxic pesticides rotenone, tebufenpyrad and pyridaben induced significant neurotoxicity by affecting mitochondrial structure and function. To further characterize the relationship between mitochondrial dysfunction and epigenetic dysregulation, we examined the effects of pesticide exposure on histone acetylation in cell and animal models of PD. Exposing N27 to rotenone (1 μM) and pyridaben (3 μM) time-dependently increased acetylation levels of the global histones H3/H4, including site-specific histone acetylation at H3-K23 and H4-K5. In a mitochondria-defective MitoPark mouse model of PD, we verified both global histone H3 and site-specific H3-K23 acetylation in the substantia nigra of MitoPark mice compared to age-matched controls. Importantly, we observed increased histone acetylation of both H3/H4 and H3-K23 in nigral lysates from postmortem human PD brains. To investigate the detailed molecular mechanisms of pesticide-induced histone hyperacetylation, we adopted the zebrafish model, which is increasingly being employed to study the epigenetic modifications relevant to human diseases. Transparent cristalline zebrafish mutant embryos, devoid of pigmentation, were exposed to various doses of rotenone at 24 h post-fertilization and harvested 4 days later. Immunostaining revealed that a 10-nM rotenone exposure induced a loss of TH neurons in the zebrafish larval brain. We intend to compare the histone global and site-specific acetylation profiles between our rotenone zebrafish model and mouse models of PD. Collectively, our findings show that mitochondrial dysfunction induces histone hyperacetylation in DAergic neu-
1307 Effects of Low-Dose Developmental Dieldrin Exposure on Neuroinflammation and α-Synuclein Aggregation in the Mouse Nigrostriatal Pathway

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Human and animal studies have shown that exposure to the organochlorine pesticide dieldrin is associated with increased risk of Parkinson’s disease. Although previous work demonstrated that developmental dieldrin exposure increases neuronal susceptibility to MPTP toxicity in male C57BL/6 mice, the mechanisms driving this increased susceptibility are not well characterized. Male mice developmentally exposed to dieldrin display enhanced MPTP toxicity compared to mice treated with MPTP alone, showing greater induction of glial fibrillary acidic protein (GFAP) and α-synuclein (α-syn) expression. This suggests that dieldrin-induced changes in neuroinflammation and α-syn may underlie increased neuronal susceptibility. Here, we tested the hypothesis that low-dose developmental dieldrin exposure induces changes in neuroinflammatory markers and α-syn, thereby increasing vulnerability of the nigrostriatal pathway to dopaminergic toxicity. Starting at 8 weeks old, male C57BL/6 mice were exposed to 0.3 mg/kg dieldrin by feeding every 3 days, continuing through adult maturing, gestation, and lactation. At 12 weeks of age, male and female pups from independent litters were sacrificed, and striatum and substantia nigra were dissected. To determine whether sex-specific changes in neuroinflammation and α-synuclein underlie male-specific enhanced vulnerability, both sexes were included in analyses. We assessed markers of neuroinflammation via target-ordered expression assays to test if developmental exposure to dieldrin led to induction of neuroinflammatory pathways in the striatum and substantia nigra. In addition, we analyzed α-syn aggregation by western blot in non-denaturing and non-reducing conditions to test whether exposure leads to changes in α-syn species. We identified changes in both systems, demonstrating that developmental dieldrin exposure produces “sub-toxic” changes in these pathways that produce a phenotype of increased vulnerability. In a parallel study, we identified sex-specific DNA methylation changes in genes related to the development and maintenance of the nigrostriatal pathway. Taken together, these data suggest that developmental dieldrin exposure leads to persistent changes in phenotype that may contribute to the development of Parkinson’s disease.

1308 Nanotherapy for Parkinson’s Disease: Nicotine-Nanoceria Prevents Neuronal Damage and Reverses Electrophysiological Function in Primary Human Dopaminergic Neurons


The pathophysiology of dopaminergic (DA) loss in Parkinson’s disease (PD) is still unclear. Lack of effective therapies maybe due to limitations in our understanding of the molecular and cellular events leading to degeneration of the nigrostriatal DA system. Here, we report that a combination of Nicotine and NanoCeria presents an outstanding case of prevention of PD progression, in a 1-methyl-4-phenylpyridinium (MPP+) induced in vitro model of PD in primary human dopaminergic neurons. A co-treatment of Nicotine and NanoCeria significantly inhibited MPP+ induced inhibition of parkin expression and prevented the aggregation of α-synuclein suggesting a regulation of protein degradation pathway. More importantly, in a functional electrophysiological analysis using Multi-Electrode Array, we report for the first time that this therapeutic combination of Nicotine and NanoCeria was not only able to prevent MPP+ induced loss of neuronal function but also was able to significantly reverse it towards normal. Our data might suggest an efficacious role of Nicotine-NanoCeria combination for the prevention of neuronal loss and restoration of neuronal function during PD progression. Additional in vivo work will be required to validate these significant findings.

1309 Function of lncRNA NR_030777 in Affecting Cell Proliferation and Apoptosis by Regulating Zfp326/Cpne5 in Nerve Cell Damage Induced by Paraquat

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Parkinson’s disease (PD) is a common age-related degenerative disease of the central nervous system. It is generally believed that PD is caused mainly by environmental and genetic factors. Previous studies have shown that paraquat (PQ) may be one of the environmental factors leading to PD, but its specific mechanism remains to be further elucidated. lncRNAs generally refer to non-coding RNA transcripts longer than 200 nucleotides and researches have shown that lncRNA plays irreplaceable roles in neurodegenerative diseases. However, the exact roles and mechanisms of lncRNAs remain unclear. In this study, we identified IncRNAs related to PD caused by PQ and clarified the biological functions and regulatory mechanisms of a specific lncRNA in nerve cell damage induced by PQ. We found changes in lncRNA expression profile of mouse substantia nigra (SN) induced by PQ using microarrays, and six of them validated by qRT-PCR and two by FISH. Bioinformatics analysis was applied to predict the functions of IncRNAs and predict the targeted genes. Among the differently expressed IncRNAs, the dysregulation of NR_030777 which is highly homologous with its gene symbol Zfp326 drew our attention. We observed that the changes of Cpn5 expression through CNC prediction and mRNA microarrays was related to the high expression of NR_030777. So we reasonably supposed that NR_030777/Zfp326/Cpne5 pathway plays a crucial part in nerve cell damage induced by PQ. In vitro, Parkinson’s disease virus infected cells used to knock down or overexpress NR_030777 expression in order to explore the regulatory mechanism and biological functions of NR_030777 in cell damage caused by PQ. We found that NR_030777 regulated the expression and stability of Zfp326 and Cpne5, and thereby regulated cell proliferation and apoptosis induced by PQ. In addition, we further discovered that by scavenging ROS by N-acetyl-cysteine (NAC), NR_030777 expression was suppressed. In summary, NR_030777 plays a protective role against neurotoxicity of PQ by regulating the expression of Zfp326/Cpne5. Our study first reveals the novel role of NR_030777 in PQ-induced nerve cell damage and provide a potential method to prevent and treat neurodegenerative diseases caused by common environmental neurotoxins such as PQ.

1310 Neurobehavioral and Neurochemical Effects of Acute to Subchronic PhIP Exposure

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Heterocyclic amines (HCAs) occur in a variety of foods, especially in the crust of charred meats. HCAs have been widely investigated as mutagens, though our published data suggest that HCAs are selectively neurotoxic to dopamine neurons, with potential relevance to Parkinson’s disease (PD). 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) is the most abundant HCA in cooked meat. Of the major primary metabolites, N-Oh-PhIP selectively affects dopamine neurons. Rodents are far less efficient than humans in metabolizing PhIP to N-Oh-PhIP due to differences in CYP1A2. Thus, we wished to test the contribution of CYP1A2 to PhIP-induced neurotoxicity using humanized mice. Here, we hypothesized that PhIP treatment in mice would lead to neurobehavioral deficits and selective dopamine neurotoxicity and that humanized mice would be more sensitive. C57BL/6 mice were exposed to acute (100 or 200mg/kg bodyweight; single dose; 8-hour exposure; males and females; n = 4-5/group) or subchronic (75mg/kg; 3x/week for 16 weeks; males; 200mg/kg bodyweight; single dose; 8-hour exposure; males and females; n = 4-5/group) PhIP. Doses and time-points were chosen from the cancer bioassay literature and our studies. Behavior was assessed through rearing and pole tests in subacute exposure groups. Brains were collected for neurotransmitter and histological analyses. Acute PhIP exposure significantly decreased striatal dopamine turnover in mice humanized for CYP1A1/2 vs. wild-type controls and wild-type exposed, indicating heightened sensitivity. Subchronic exposure significantly decreased motor function as evidenced by decreased rearing and increased pole test descent time, with behavioral changes detectable beginning after 11 weeks of treatment. Subchronic PhIP-treated mice showed significantly increased striatal dopamine levels, with no significant changes in other neurotransmitters. Turnover changes were not detected, suggesting the effect is acute. There were no detectable changes in striatal dopamine terminal density or oxidative damage markers in either sex, suggesting a lack of overt neuropathology. In conclusion, our studies in mice support findings in other model systems showing that PhIP selectively targets dopaminergic neurotransmission and that PhIP metabolism may have a critical role in neurotoxicity. Future studies will add male and female mice exposed from subacute to chronic time-points to determine potential relevance to human PD.
Environmental toxiants that cause mitochondrial dysfunction, such as the organic pesticide rotenone, and the common herbicide paraquat, are associated with elevated Parkinson’s disease (PD) risk. A heavily used industrial solvent, trichloroethylene (TCE), also causes mitochondrial toxicity, and is a ubiquitous environmental contaminant. Occupational TCE exposure is linked to the development of PD (odds ratio (OR): 6.1; 95% CI). Similarly, high doses (900-1,000 mg/kg) of TCE administration to rodents results in dopaminergic neurodegeneration. New data from our lab indicates that rotenone, paraquat, and TCE interact with PD susceptibility genes, notably, causing the activation of the protein LRRK2 (leucine-rich repeat kinase 2) in wildtype human embryonic kidney (HEK) cells, which could be blocked by a selective LRRK2 inhibitor (GNE-7915). As LRRK2 is the most commonly inherited mutation associated with familial PD, this evidence suggests that a gene-environment interaction exists between LRRK2 and common environmental mitochondrial toxicants.

Aberrant LRRK2 activation leads to pleiotropic cellular dysfunction, such as disruption of vesicular trafficking and autophagy, accumulation of phosphorylated alpha-synuclein, and neuroinflammation; all of which are mechanisms hypothesized to precede dopaminergic neuron degeneration in PD. As TCE is a widespread environmental contaminant, we postulated that relatively low levels of TCE exposure in aged rats may induce LRRK2 activation in dopaminergic neurons and increase risk of a Parkinsonian phenotype. Using aged Lewis rats (12 mo) we administered 200 mg/kg TCE (oral gavage) or vehicle (olive oil) daily for 6-weeks. Animals receiving TCE displayed a moderate loss of dopaminergic neurons within the substantia nigra, which correlated with a significant increase in LRRK2 kinase activity in surviving cells. In addition, we observed marked deficits in endolysosomal trafficking and function, concomitant with accumulation of toxic forms of alpha-synuclein (phospho-Serine129) within dopaminergic neurons. Together, these data suggest that LRRK2 activation by environmental TCE may be a novel gene-environment interaction, resulting in early pathological changes within susceptible individuals to increase PD risk.

The etiology of neurodegenerative diseases such as Parkinson’s (PD) and Alzheimer’s disease is unknown but likely linked to combinatorial interactions between genetic risk factors, environmental stressors including viruses, and environmental neurotoxins. Our understanding of the environmental links to PD and related neurodegenerative disorders remains extremely limited, emphasizing the need for better animal models in which to test interactions between environmental toxins and genetic backgrounds. Current animal models emphasizing the need for better animal models in which to test interactions between environmental toxins and genetic backgrounds. Current animal models focusing on the use of neurotoxins and transgenic mice may show loss of neurons but often lack other key hallmarks of these diseases, such as neuroanatomical specificity, progressive neuronal loss, glial activation and protein aggregation. Notably, certain neurotropic mosquito-borne alphaviruses, such as Western equine encephalitis virus (WEEV), can target the midbrain, or vago, or vehicle (olive oil) daily for 6-weeks. Animals receiving TCE displayed a moderate loss of dopaminergic neurons within the substantia nigra, which correlated with a significant increase in LRRK2 kinase activity in surviving cells. In addition, we observed marked deficits in endolysosomal trafficking and function, concomitant with accumulation of toxic forms of alpha-synuclein (phospho-Serine129) within dopaminergic neurons. Together, these data suggest that LRRK2 activation by environmental TCE may be a novel gene-environment interaction, resulting in early pathological changes within susceptible individuals to increase PD risk.

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Evidence indicates that complex gene-environment interactions underlie the incidence and progression of Parkinson’s disease (PD). Neuroinflammation, characterized as a well-characterized feature of PD, is widely believed to exacerbate the neurodegenerative process. Environmental toxicants associated with PD, such as pesticides and heavy metals, can cause cellular damage and stress potentially triggering an inflammatory response. Inflammasomes are intracellular protein complexes that contain pattern recognition receptors capable of initiating and propagating inflammation in response to toxicants, pathogens, and non-pathogenic cellular damage and stress. Our laboratory has characterized the NLRP3 inflammasome in toxicant-based animal models and PD patients. Long-term intragastric exposure to the PD-associated pesticide and mitochondrial toxin rotenone results in Nlrp3-dependent systemic and neurologic inflammation and Nlrp3 mice are protected from rotenone-induced nigral cell loss. Similarly, in MPTP treated animals, we observe increased sparing of nigral neurons in Nlrp3 homozygous mice with a dramatic reduction in nigral microgliosis. In PD patients, histologic studies revealed elevated NLRP3 expression in mesencephalic tissues. Analysis of exome sequencing data for genetic variation of NLRP3 in PD identified the rs7525979 variant associated with a significantly reduced risk of developing PD. We subsequently characterized this polymorphism and found that the rs7525979 variant caused a severe disruption of the NLRP3 protein lifecycle likely resulting in inflammasome inactivation. Further characterization of how inflammasomes may function in PD is a high priority because most PD cases are sporadic, supporting the widely-held belief that environmental exposure is a major factor in disease initiation and progression. Inflammasomes may represent a common mechanism that helps to explain the strong association between exposure and PD by mechanistically linking environmental toxicant-driven cellular stress with neuroinflammation and ultimately cell death.

Using Human Cells Based High-Throughput Neuronal-Schwann Cells Culture System

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Currently, high-throughput screens (HTS) for chemotherapy-induced peripheral neuropathy are lacking, because in vitro systems fail to reliably mimic the side effects of chemotherapeutics, the physiological function of the cells. HTS based on neurons alone lacks the physiological complexity of the nervous system, as it does not account for the contribution of glial cells in neuropathy. To overcome this, we have characterized a novel human cells-based coculture system comprising of iPSCs-derived sensory neurons (hSNs) and primary human Schwann cells (hSCs). HTS platforms, such as high content imaging systems (HCS) and multielectrode arrays (MEA) can provide more relevant biological information than standard biochemical assays. We first compared a range of densities and ratios of hSNs to hSCs to reveal that a 4:1 ratio induced the fastest electrical maturity in terms of firing rate using the MEA system. A coculture of 30,000 hSNs to 7,500 hSCs became electrically active on day 6, while hSNs alone required densities of 70,000 neurons to become electrically active on day 6. MEA experiments showed the benefits of the coculture system in terms of faster, more consistent electrical maturation as well as a reduced number of hSNs resulting in reduced cost of screening. Furthermore, we used the same 4:1 ratio for evaluating dose responses using HCS system for two chemotherapeutic drugs, Paclitaxel and Oxaliplatin. Automated high-throughput neurite outgrowth and cell count analysis showed that the cocultures were less sensitive to drugs compared to monocytes in terms of overall neurite length, number of branches, number of processes, and total number of cells. Paclitaxel showed a toxic response only with hSCs in the coculture as opposed to monocytes. While Oxaliplatin did not show differences in neurite outgrowth parameters between mono- and cocultures, viability of neurons was still found to be different. The IC50 and LD50 concentrations were found to be significantly different between cocultures and monocytes across all parameters. These differences in sensitivity show the importance of the presence of glia along with neurons in high-throughput assays to capture biological complexity. In summary, both the MEA and HCS experiments demonstrated that a high-throughput neuronal-glial coculture system can be a more effective tool for assessing the safety of potential therapeutics results.

Expression of Drug-Metabolizing Enzymes in Cochlea: Implications for Ototoxicity of Analgesics

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Acetaminophen (APAP) and non-steroidal anti-inflammatory drugs (NSAIDs) are the most popular drugs in the US, but can cause hearing loss. Epidemiological studies indicate that chronic use of APAP and NSAIDs over the course of years increases risk of hearing damage. Additionally, numerous reports of rapidly progressive hearing loss in patients taking APAP/opioid combinations have been published. Importantly, though, little is known about the mechanisms of ototoxicity of these drugs. In other tissues, the toxicity of APAP and some NSAIDs is dependent on drug metabolizing enzymes (DMEs) and transporters. In particular, cytochrome P450 (Cyp) enzymes catalyze formation of toxic reactive intermediates, while UDP-glucuronosyltransferases (UGTs), sulfotransferases (Sults), and transporters promote drug clearance. However, expression of DMEs and transporters in cochlea has never been systematically studied. We created a PCR panel to compare expression of major isoforms of these enzymes in mice between cochlea and liver, an organ with high expression of DMEs. The panel includes 11 Cyps, 8 UGTs, 3 Sults, and 8 transporters. The specific DMEs and transporters were chosen because they account for metabolism of more than 70% of all clinically-relevant drugs. Data from PCR was confirmed by enzyme kinetics. Expression of Cyp was low in the cochlea compared to liver, with the exceptions of Cyp1a1, Cyp1b1, Cyp2c65, and Cyp2c66, which had similar levels of expression. All UGTs and Sults were also lower in cochlea than in liver, except for UGT1a3 and Sult2a1. Interestingly, expression of most transporters was low, with two major exceptions: Abcc2/8bcrp expression was similar between cochlea and liver, while expression of Mdr1/P-gp, which is thought to be highly expressed in cochlea, was barely expressed in other tissues. On the other hand, Bcrp expression was significantly higher in cochlea compared to liver, while expression of Mdr1/P-gp, which is thought to be highly expressed in liver and poorly expressed in neuronal tissue, was 3-fold greater in cochlea.

Organophosphorus nerve agents exert their peripheral and central toxicity by deactivating acetylcholinesterase (AChE), leading to an accumulation of acetylcholine (ACh) in the synaptic cleft. Prolonged residency of ACh can overstimulate nicotinic acetylcholine receptors (nAChRs) in diaphragm muscle, resulting in respiratory failure. The oxime pyridine-2-aldoximochloride (2-PAM) has historically been used to re-activate AChE following nerve agent exposure. A second oxime, 1,1’-methylenebis(4-(hydroxyimino)methyl)pyridinium (MMB4), is currently under development as a more potent and more promiscuous replacement for 2-PAM. However, preclinical animal studies have demonstrated that MMB4 has an exacerbated toxicity in rabbits at doses exceeding 200 mg/kg MMB4. One possible mechanism for MMB4 toxicity is via antagonism of nAChR due to its molecular similarity to ACh. Patch-clamp electrophysiology was used to compare agonist-evoked currents in the presence of MMB4 or 2-PAM on recombinant human α1 and α7 nAChRs, as well as α1 nAChR in dissociated mouse flexor digitorum brevis (FDB) muscle fibers. Preliminary data suggest that both oximes antagonize human and mouse α1 and α7 nAChRs, with 2-PAM acting as a more potent inhibitor in all cases. We anticipate that these studies will contribute to our understanding of oxime toxicity at neuromuscular junctions and define important safety considerations when screening future nerve agent antidotes.
Changes of CaMKII/CREB Signaling in NAc during the Acquisition, Extinction, and Reinstatement Phase of the Nicotine-Induced Conditioned Place Preference in Rats

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The Ca2+-calmodulin-dependent protein kinase II(CaMKII) and cAMP response element binding protein(CREB) are involved in nicotine reward and withdrawal. In the current study, we investigated the roles of CaMKII/CREB signaling in the nucleus accumbens(NAc) in the three phases (acquisition, extinction, and reinstatement) of the nicotine-induced conditioned place preference(CPP). CPP was induced by the subcutaneous injection of nicotine(0.5mg/kg) at seven dosing periods, and 4 d saline training extinguished CPP. CPP was reinstated by the injection of nicotine(0.2mg/kg) after three dosing periods. During the acquisition phase of CPP, the levels of CaMKII and pCaMKII were significantly decreased. However, these changes disappeared in the extinction phase, indicating the levels of CaMKII and pCaMKII return to normal. The level of CREB was significantly decreased in the extinction phase. There were no significant differences in the reinstatement phase of CPP. The results show that CaMKII/CREB signaling in the NAc may play critical roles in nicotine addiction and withdrawal.

Exposure to Bisphenol A Impairs Mitochondrial Function and Alters the Activity of Antioxidant Enzymes in Human Neuroblastoma Cells


The potential human health risk of Bisphenol A (BPA) is considered a major public health concern. Exposure to even a low dose of BPA has been linked to diverse negative pathological, cellular, and molecular effects. Neurodegeneration has increasingly been associated with mitochondrial dysfunction and inhibition of the electron transport chain. Neurons require high levels of energy in order to operate. Therefore, either exceedingly high demand for ATP or diminished production of ATP can affect normal neuronal function and the level of brain stress encountered during the development of neurodegeneration. In addition, the production of ATP in these neurons may be reduced due to mitochondrial dysfunction. Mitochondrial membrane potential is an important parameter of mitochondrial function used as an indicator of cell health. To address the cellular and molecular mechanisms that might underlie BPA-induced brain damage, we extended our previous studies of BPA-induced neural toxicity by further investigating the mitochondrial dysfunction and antioxidant enzymes activity in this process. Human neuroblastoma SH-SY5Y cells were exposed to BPA at a concentration range of 0.01 to 50 µM for up to 24h. Cell viability, mitochondrial membrane potential, and the activity of antioxidant enzymes (glutathione reductase, glutathione peroxidase, and catalase) were evaluated. Exposure to BPA for 3h resulted in a significant decrease of mitochondrial membrane potential similar to CCCP, the mitochondrial uncoupler positive contrast, no effect was observed on the activity of catalase after exposure to BPA. The activity of glutathione reductase and glutathione peroxidase was significantly reduced at concentrations of 10µM and 50µM BPA. In contrast, no effect was observed on the activity of catalase after exposure to BPA. These results suggest that loss of mitochondrial membrane potential, depletion of the cellular levels of ATP, and alteration of the capacity of antioxidant enzymes may play a contributory role of BPA toxicity and that BPA adversely results in neurodegeneration. The findings of this study confirm that increases in BPA concentration reduce ATP levels in neurons inducing an energy crisis that drastically interferes with the neurons’ ability to mount effective defenses against the toxic action of BPA. Supported by Title III.

Ethanol Increases Manganese-Induced Spatial Learning and Memory Deficits via Oxidative/Nitrosative Stress Induced p53 Dependent/Independent Hippocampal Apoptosis

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The diet is a major route of manganese (Mn) exposure for humans. The diet is a major route of manganese (Mn) exposure for humans. Evidence of histopathology was used as the standard for CNS toxicity diagnosis, with special staining applied in some cases for improved detection. Additional in-life endpoints included functional observational battery (FOB) and MRI imaging. In general, these neurotoxicants exhibited a steep dose response curve and variability in CNS toxicity response and mortality. Although most studies produced expected CNS injuries by the standard of histopathology, some gave rise to more complicated results, in which severe mortality was observed without visible histopathological findings in the CNS. In the latter cases, FOB often provided sufficient sentinel signals in a dose-response manner even in the absence of general clinical signs. On the other hand, MRI imaging with certain algorithms provided a noninvasive approach to assessing the neuronal and axonal injuries correlating with histomorphological changes. With this presentation, we will discuss the challenges and pitfalls in animal model development and potential ways to optimize the process, compare findings in FOB and MRI imaging with histopathology in relation to different treatments, and discuss the utility of the integrated endpoints in potential preclinical CNS toxicity screening.
concentrations of 400 μM, did not produce seizures. In 14 DIV rat cortical neurons or 14 DIV rat hippocampal neurons. In the 14 DIV GlutaNeuron/Astrocyte co-culture, however, there was a robust response at 31.6 μM, with decreasing intensities at lower concentrations in a dose response manner. At 31.6 μM, pilocarpine caused up to 2-fold decreases in firing rates, 3-fold increases in percent isolated spikes (spikes occurring outside of bursts) and S to 10-fold increases in median/mean ISI and median ISI (indicators of burst structure deterioration) in the 14 DIV GlutaNeuron/Astrocyte co-culture. In subsequent experiments, we restated pilocarpine in the rat cortical neurons and hippocampal neurons at 21 DIV, which produced a much more robust response. The changes in the rat cortical neurons included 2-fold decreases in the number of spikes in bursts and 2-fold decreases in the median ISI, both responses indicating a deterioration in burst organization. We also observed a breakdown in network synchrony. This pattern was consistent with the iPSC neuron response observed at 14 DIV. Alternatively, the 21 DIV rat hippocampal neurons responded with an increase in regularity characterized by changes in endpoints such as 3-fold increases in the number of spikes in bursts, 3-fold increases in burst duration and 2-fold decreases in the MAD burst spike number, which indicates an increase spike train regularity. When changes in muscarinic receptor expression over time were measured, expression changes in maturing cells were the cause of the significant differences. In conclusion, we have identified different neuronal cell types is important for determining whether a model is correct for identifying liabilities associated with specific receptors.

1324 Role of Microglial Activation and Neuroinflammation in Neurotoxicity of Acrylamide, an Environmental Soft Electrophile

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Acrylamide (ACR) is widely used in the production of polymers or gels and is also formed when carbohydrate-rich foods are cooked at high temperature. Neurotoxicity of ACR has been reported in humans and experimental animals, while the underlying mechanism remains elusive. The present study aims to investigate the role of microglial activation and neuroinflammation in the neurotoxicity induced by ACR exposure. First, male Wistar rats were exposed to ACR by gavage at 0, 0.2, 2, or 20 mg/kg BW, 7 d/week for 5 weeks. At the end of the exposure, rats were decapitated and the cerebral cortex was dissected out. Expression of inflammatory, neurotoxic, and microglial markers in the cerebral cortex were examined by real-time quantitative reverse transcription (qRT-)PCR, Western blot or immunohistochemistry. Secondly, BV2 microglial cells were treated with ACR at different dose (0, 5, 50, or 500 μM), for different time duration (0-36 h). MTS assay was performed to evaluate effects of ACR on cell viability. Expression of inflammation-related genes was examined by real-time qRT-PCR. In vivo studies showed that 5-week ACR exposure at 20 mg/kg BW upregulated mRNA levels of cytokines IL-1β and IL-6 in rat cerebral cortex; and ACR exposure at 2 mg/kg BW significantly upregulated IL-18 level. In vitro studies showed that 5-week ACR exposure at 20 mg/kg BW upregulated mRNA levels of cytokines IL-1β and IL-6 in rat cerebral cortex; and ACR exposure at 2 mg/kg BW significantly upregulated IL-18 level. Western blot showed that IL-1β was increased after exposure to ACR at 0.2 and 2 mg/kg BW, and IL-18 was increased by exposure to ACR at 20 mg/kg BW. ACR exposure at 2 mg/kg BW upregulated mRNA level of microglial markers CD11b and CD40. Immunohistochemistry showed that exposure to ACR at 2 or 20 mg/kg BW increased CD11b/c positive microglial area and/or length of processes, suggesting microglial activation after ACR exposure. The NLRP3 inflammasome-related genes, including NLRP3, Caspase 1 and ASC, which regulate production of IL-1β and IL-18, were upregulated by ACR exposure at 2 mg/kg BW. In vitro studies showed that BV2 cell viability measured by MTS assay was decreased by exposure to ACR only at 2000 μM or above. ACR exposure increased mRNA levels of cytokine IL-1β and IL-18 in BV2 cells in a time- and dose-dependent manner. Expression of iNOS, which is an inflammatory marker, was significantly upregulated by ACR exposure. The above in vivo and in vitro results suggest that ACR exposure induces neuroinflammation, including microglial activation and upregulation of cytokine expressions.

1325 Development of a Neurotoxicity Assay That Is Tuned to Detect Mitochondrial Toxicants

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Mitochondrial toxicity is one of the major reasons for drug withdrawals. In particular, many neurotoxicants affect energy metabolism. Both animal models and cell-based test methods often fail to predict mitochondrial effects and neurotoxicity independent of neuronal activity. Thus, there is a need for improved sensitivity in this area. LUHMES cells are conditionally immortalized human neuronal precursors that can be quickly differentiated to fully mature dopaminergic neurons. They have been routinely used for the NeurTox assay to screen and characterize neurotoxicants. Using this test method, cells have been exposed here to a panel of 30 test chemicals for 24 h. Data (cell viability and overall neurite area) were acquired, after live cell staining, by high content imaging microscopy coupled to a fully automated data processing pipeline. In parallel, metabolic data, such as ATP content, central carbon metabolite levels, mitochondrial oxygen consumption and lactate production were monitored. Experiments were run in medium, containing either 18 mM glucose or 18 mM galactose as main carbohydrate source. Mitochondrial toxicity was predicted by the ratio of the EC25 values (for neurite outgrowth) in glucose medium (cells little dependent on mitochondria) vs. galactose medium (cells highly dependent on mitochondria). The panel of chemicals contained at least 5 inhibitors for each mitochondrial respiratory chain (MRC) complex, and also chemicals not affecting mitochondrial respiration. We found that inhibitors of MRC complexes I, II, IV and V were detected more sensitively in galactose medium, and their EC25 ratio (glucose/galactose) was >2. Uncouplers, complex II inhibitors and non-mitochondrial toxicants had EC25 ratios close to 1. Therefore, the modified NeurTox assay represents a novel and more sensitive method to detect neurotoxicants. Moreover, it pinpoints chemicals that inhibit various functions of the MRC (except for complex II).

1326 TSPO Gene Dosages Decrease Nox2 Subunit Gene Expression in Microglia: Implications for Regulation of ROS in the CNS

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Translocator protein 18 kDa (TSPO) is a biomarker of neuroinflammation and brain injury that has been extensively used in preclinical and clinical studies. TSPO is expressed in glial cells, specifically microglia and astrocytes, and levels increase rapidly following CNS insult. Similar to TSPO, NADPH Oxidase (Nox2) is a protein that is highly expressed in microglia and it is a major source of ROS production in the CNS. Previous studies from our laboratory have shown a direct protein-protein interaction between TSPO and NOX2 in primary microglia. We have proposed that the functional significance of this novel interaction may be to modulate reactive oxygen species (ROS) production in microglia with important implications to brain inflammation and neurodegeneration. In this study, we used global TSPO knockout (KO) mice to examine the relationship between TSPO and NOX2. Mice heterozygous for TSPO were obtained from Helmholtz Zentrum Munich (GMC), Germany as part of the International Mouse Phenotyping Consortium (IMPC) and INFRAFRONTIER /European Mouse Mutant Archive (EMMA). We used these animals to generate TSPO-wildtype (WT), -heterozygous (HET), and - knockout (KO) primary microglia and examined TSPO and Nox2 subunit gene expression. Quantitative real-time reverse transcriptase PCR (qRT-PCR) was used to assess mRNA expression for Tspo and the Nox2 subunits gp91phox, p22phox, p40phox, p47phox, and Rac1. We also measured Vdac mRNA expression as a mitochondrial marker since TSPO is highly expressed in mitochondria and heme-oxidase 1 (HO-1) which has anti-oxidative properties. As expected, we found a TSPO gene dosage effect on mitophagy in Nox2 subunits gp91phox, p22phox, and p40phox. Expression of gp91phox, p22phox, and p40phox were significantly decreased in a TSPO gene dose-dependent manner with no effect on p47phox, p47phox, and Rac1. No significant effect of TSPO gene dosage was found on Vdac or Ho-1 mRNA expression. Future studies will examine the effect of TSPO gene dosage on other subunit protein expression and NOX2 enzymatic activity. These novel findings strongly suggest that TSPO specifically regulates the transcription of key NOX2 subunit genes with significant implications for the modulation of ROS production and neuroinflammation in the CNS. NIEHS grant number ES007062-19 to TRG.

1327 Effects of New Psychoactive Substances on Neuronal Activity in Vitro Measured Using Microelectrode Arrays (MEAs) in Rat Primary Cortical Neurons and Enzymatic and Prolonged Exposure and Washout

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The use and number of new psychoactive substances (NPS) is increasing even though pharmacological and toxicological knowledge is limited. Most data on mechanisms of action is acquired by studying specific targets, like monoaminergic transporters and receptors, after or during acute exposure. While information on acute effects is useful to determine potency and possible structure-activity relationships, exposure scenarios that more closely resemble human exposure may further increase health risk assessment. Therefore, we
determined the effect of 11 illicit drugs and NPS on neuronal activity using different durations of exposure (30 min and 4 h). Rat primary cortical cultures were grown on microelectrode arrays (MEAs) and exposed to amphetamine-type stimulants, cathinones, hallucinogenic phenethylamines, pergazines or cocaine. Reversibility of effects was determined after a washout period of 19 h, i.e. 24 h after the start of exposure. During acute exposure, all drugs decreased concentration-dependently inhibited neuronal activity. Prolonged exposure did not further increase inhibition of neuronal activity compared to acute exposure. After 19 h washout, effects of methamphetamine, cocaine, methylene and BZP on neuronal activity were fully reversible. For all other compounds, a right-shift in potency was seen indicating (partial) recovery following washout. Moreover, neuronal networks exposed to MAPM and MDMA at concentrations relevant for human exposure did not show a full recovery of neuronal activity. Interestingly, neuronal activity increased following washout of human-relevant concentrations of methylene. Recovery measurements can have an added value in hazard characterization of emerging NPS by identifying substances that may not be the most potent inhibitors of neuronal activity following acute exposure, but may be more harmful following prolonged exposure and washout due to poorly reversible effects.

2,4,6-Tribromophenol Differentially Regulates ABC Transporters In Vivo and Ex Vivo in Rat Brain Microvessels


2,4,6-Tribromophenol (TBP, CAS No. 118-79-6) is a brominated chemical used in the production of flame retardant epoxy resins and as a wood preservative. TBP is found in marine environments, where it is incorporated in shellfish that may be consumed by predatory fish. It is most commonly detected 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 microvilli 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, ABCC2). We measured TBP-dependent changes in transport activity in freshly isolated rat brain microvessels (adult male Sprague-Dawley rats) using a confocal microscopy-based assay to measure the steady-state luminal accumulation of a transporter-specific fluorescent substrate after oral administration in vivo or direct application to ex vivo preparations. We found TBP exposure resulted in a time- (1-4h) and dose- (1-1000 nM) dependent decrease in P-gp and BCRP transport activity ex vivo, and a dose- (0.4-10 µmol/kg, 4h) dependent increase in vivo. We saw no change in MRP2 transport activity under identical conditions, indicating selectivity and capillary integrity. Our work is meaningful because changes in transporter activity in the blood-brain barrier can impact immunopathogenesis and increase CNS exposure to potentially toxic endobiotics and xenobiotics from the brain. This research was supported in part by the Intramural Research Program of NIH/NIC (Project ZIA BC 011476).

Developing Highly Accurate Computational Models for Neuronal Targets


The ability to mechanistically predict whether compounds will or will not target important protein receptor(s) is a major goal of toxicology. Thus, we sought to build such models for major neuronal targets. We mined public data sources (ToxCast, CHEMBL, BindingDB and ZINC), together with the scientific literature for compounds that did or did not interact with the nicotinic and muscarinic acetylcholine receptors, acetylcholinesterase, the GABA-A and B receptors as well as the serotonin and glycine cys-loop receptors. We developed machine-learning algorithms in KNIME using structural fingerprints and two-dimensional identification of common structural motifs (scaffolds) to screen compounds for interaction with these receptors. For targets with a sufficient number of active compounds, the fingerprint and scaffold-based prediction models were able to predict a positive outcome with high sensitivity (>80%). Exquisite sensitivity (98.4-100%) and balanced accuracy (82.6-100%) statistics were observed for data-rich targets such as the cholinergic system. The high sensitivity of these models correlated with high negative prediction values (84.7-99.8%), underscoring the confidence with which novel compounds can be aligned with these neuronal targets. By iteratively building our models with 1-90% of the compiled data, we show that <20% of data are needed to yield >75% balanced accuracy, suggesting that these models will not change for the foreseeable future. In conclusion, we demonstrate the feasibility of using computerized workflows to mine public data and develop accurate positive and negative prediction models for important neuronal targets. Due to their implementation within KNIME, these models can be used to rapidly screen in vivo datasets and provide mechanistic insights into the modes of action for substances of interest.

High Fat Diet Consumption Results in Bioenergetic Crisis and Oligodendrocyte Loss in the Spinal Cord in a NAD-Dependent Fashion


Metabolic syndrome is a prevalent co-morbidity in both spinal cord injury and multiple sclerosis patients, so a better understanding of how a high fat diet (HFD) contributes to oligodendrocyte loss has the potential to highlight new therapeutic targets. Mice consuming a HFD for 12 weeks had a significant loss of oligodendrocyte lineage cells in the dorsal column of the spinal cord and corpus callus of the brain as revealed by immunohistochemistry. Moreover, impaired spinal cord mitochondrial function was observed in chronic HFD-fed mice by decreased oxygen consumption rates (OCR), significant depletion of mitochondrial (ATP) cycle intermediates and changes in genes and proteins related to mitochondrial biogenesis and quality control processes. In oligodendrocyte progenitors, increased mitochondrial fragmentation and a reduced OCR were observed when exposed to saturated fat such as palmitic acid. Astrocytes, however, significantly increased OCR following exposure to palmitic acid and increased expression of pro-inflammation markers. In HFD-induced oligodendrocyte lineage cell loss includes an impairment of NAD+-mediated mitochondrial dynamics and bioenergetics. Importantly, we show that pharmacological inhibition of a NAD+-degrading enzyme, CD38, with 78c can overcome the dysmyelinating effects of lyssolecithin and palmitic acid in ex vivo cerebellar brain slice cultures. Together, these findings suggest that changes in the spinal cord in response to consumption of a HFD creates an environment less conducive to neural repair processes in neurological disorders by impairing mitochondria function and thus oligodendrocyte survival.

Oral Administration of Citronellal for Eight Weeks Does Not Produce Large Changes in Peripheral Nerve Function or Somatosensory Evoked Potentials

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Citronellal is a monoterpenic oil that has been reported to have anti-nociceptive properties in mice when given alone to produce the amplitude of compound action potentials in the sciatic nerve of an ex vivo frog preparation. These findings suggest that citronellal has the potential to alter peripheral nerve function. The physico-chemical properties of citronellal indicate it has the potential to react with cellular proteins through imine formation. We treated adult male Long-Evans rats with an oral gavage of 0 (corn oil vehicle), 9.5, 11.6, or 16.8 mg/kg/day citronellal for 8 weeks. Behavioral observations (gait, open field activity, arousal, foot splay, grip strength, rearing) were made each week during treatment. Nerve excitability testing was performed using recordings from the tail nerves (motor and mixed) and sciatic (motor) nerve during the 6th week. Compound nerve action potentials (CNAP) and nerve conduction velocity (NCV; tail nerves), and somatosensory evoked potentials (SEPs) were recorded over the cortex and cerebellum during the 8th week of treatment. Treatment with citronellal did not alter the animal’s weight gain over the 8 weeks. No changes were observed for gait, open field activity, arousal, hindlimb foot splay, or rearing. There was evidence of decreased motor strength, but the results were not dose- or time-related. Nerve excitability testing of tail motor nerves did not indicate changes in nerve function. However, nerve excitability testing of the sciatic nerve suggested increased thresholds following a hyperpolarizing pulse, possibly due to greater membrane hyperpolarization associated with decreased K⁺ conductance. No changes in tail mixed nerve SEPs were indicated. No changes in tail CNAPs, NCV, or SEPs from the cortex or cerebellum were indicated. Our data
suggest that treatment with citronellal (over this dose range and duration) did not result in large changes in peripheral nerve, motor, or somatosensory function. These results do not preclude anti-nociceptive properties after acute treatments. This is an abstract of a proposed presentation and does not necessarily reflect US EPA policy.

1332 Propranolol as a Novel Treatment for Gulf War Illness in a Preclinical Mouse Model
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Gulf War Illness (GWI) is a multi-symptom, neuro-immune-based disorder that presents with features similar to sickness behavior. Unfortunately, current treatments for GWI tend to focus on managing symptoms as opposed to addressing the underlying cause of the illness. Using a preclinical mouse model, we have found that GWI is associated with an exacerbated neuroinflammatory response to immune challenge, like lipopolysaccharide (LPS) exposure, and the activation of microglia. Interestingly, β-adrenergic antagonism has been found to inhibit microglial activation and its associated release of inflammatory cytokines. Here, we tested the therapeutic potential of the beta-blocker propranolol in our established mouse model of GWI. In this model, mice are exposed to the stress hormone corticosterone (CORT; 200 mg/L) in the drinking water for 7 days followed by a single injection of diisopropyl fluorophosphate (DFP; 4 mg/kg, i.p.) to model the “in theater” conditions of high physiological stress and potential nerve agent exposure. This is then followed by periodic administration of CORT for 7 days every other week to a total of 5 weeks with a systemic LPS challenge (0.5 mg/kg, s.c.) on the final day. Propranolol (20 mg/kg, i.p.) was given during or outside of CORT exposure. Mice were sacrificed 6 hours after LPS challenge and brain cytokine mRNA expression was evaluated by qPCR. We found that propranolol significantly reduced the neuroinflammation instigated by the GWI exposure model when given during CORT exposure. In particular, treatment reduced cytokine expression in the GWI exposure group to levels comparable to CORT-LPS, which models a normal response to inflammatory challenge. These initial studies indicate the potential for propranolol to treat the underlying neuroinflammation associated with GWI and to return veterans to a healthy neuroimmune functional state. Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention.

1333 Functional Characterization of Neural Network Activity in Human iPSC-Derived Neuron/Glial Co-Cultures

The increasing amount of chemicals with human exposure, and the accumulating evidence for inter-species differences regarding adverse effects on the CNS, call for scalable in vitro neurotoxicity screening tools using human cells. Generating neuronal cells through differentiation from induced pluripotent stem cells (iPSCs) carries a great potential to overcome the inaccessibility of human primary tissue and accelerate cell-based assays. In combination with multi-electrode array (MEA) readouts, neural activity in response to chemical compounds can be quantified allowing neurotoxicity screening. However, the assessment of neuroactive effects in human-derived cell-based assays remains challenging due to cell type variability and poorly defined baseline physiology. Here, we describe a new screening platform using highly functional neural cultures with defined cell ratios consisting of excitatory and inhibitory neurons that were separately generated by direct conversion from human iPSCs (NeuCyte SynFire®16), as well as primary human astroglial cells. The reduced complexity of this iPSC-derived co-culture system, compared to primary rodent cultures, enables a detailed molecular and functional characterization to define its applicability domain and develop screening assays. Therefore, we conducted comprehensive transcriptome profiling at different maturation time points of the co-cultures and tested altered neuronal firing, bursting and synchrony metrics in response to a set of 15 agonistic and 15 antagonistic tool compounds targeting neuronal signaling (e.g. GABA, AMPAR, NMDAR, ACHR, D1/2R, and 5-HTR) on MEAs. We then correlated dose-dependent responses with expression patterns at the different co-culture time points to determine sensitivity, specific neuroactivity profiles and potential assay windows for interference with these pathways. Furthermore, we confirmed specific responses by patch clamping of matched neuronal/glial co-cultures. This unique physiological characterization of NeuCyte’s human iPSC-derived neuron/glial co-cultures as baseline and reference for multiple neurotoxicity testing applications.

1334 Nanomolar Tetramethylpyrrole Alters Ca2+ Dynamics in Cortical Neuronal Networks by Selective Modification of Rydnome Receptors and Micromolar is Neurotoxic Due to SERCA Pump Inhibition
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Naturally synthesized marine organohalogenes (MOH) and their anthropogenic homologs produced as disinfection byproducts (DBP) are an emerging environmental health concern because several have been identified to exhibit potent biological activities in model systems, including cytotoxicity, genotoxicity, carcinogenicity and developmental toxicity. The molecular mechanisms underlying toxicity are poorly understood. Recently we discovered that several specific MOH and DBP measured in environmental and biological samples, including halopyrroles, halobipyrroles, haloindoles, and hydroxylated polybrominated diphenylethers directly modify rydnone receptors and SERCA pump activity, two key proteins anchored within sarcoplasmic/endoplasmic reticulum (SR/ER) that work in physiological opposition to tightly regulate net ER/SR Ca2+ dynamics and thereby shape meaningful Ca2+-dependent cellular processes. Using intact HEK293 cells null for rydnone receptors (RyrRs) expression and those that stably express Ryr1, we demonstrate that tetramethylpyrrole (TDP) selectively sensitizes Ryr1 channels to caffeine-triggered Ca2+ release only in Ryr1-expressing cells, TBP at higher concentrations also depletes of SR/ER Ca2+ stores in both null and Ryr1 expressing cells commensurate with its lower potency to inhibitory SERCA in biochemical assays. Exposure of primary neuronal/glial co-cultures derived from newborn mice shows that TBP inhibits the frequency and amplitude of spontaneous Ca2+ oscillations (IC50 = 246 and 426nM, respectively), whereas >1µM produces a sustained rise in cytoplasmic Ca2+. Subchronic (24HR) exposure to TBP caused loss of neuronal/gial viability using the MTT assay (EC50 =12.4µM). These results show that nM TBP selectively targets Ryr-mediated Ca2+ dynamics in a manner that has been shown to affect neurodevelopment, whereas low-µM exposures cause overt neurotoxicity, likely mediated by the combination of Ryr activation and SERCA inhibition. Supported by NIH grants ES030318 and ES014901.

1335 Tetramethylphenol A Alters ABC Transport at the Blood-Brain Barrier

Tetramethylphenol A (TTPA, CAS No. 79-94-7) is a brominated flame retardant (BFR) used in 90% of epoxy coated circuit boards. Exposures to TTPA can disrupt mitogen-activated protein kinase (MAPK), estrogen, thyroid and peroxisome proliferator-activated receptor (PPAR) signaling pathways. Since these pathways also regulate transporters of the CNS barriers, we sought to determine the effect of TTPA on the expression and activity of three major ABC efflux transporters of the blood-brain barrier (BBB). Using a confocal based assay, we measured the ex vivo and in vivo effects of TTPA on P-glycoprotein (P-gp), Breast Cancer Resistance Protein (BCRP), and Multi-Drug Resistance Protein 2 (MRP2) transport activity in rat brain capillaries. We found TTPA (1-1000 nM) had no significant effect on MRP2 transport in either sex. However, low concentrations of TTPA (1-100 nM) significantly decreased BCRP transport activity in both sexes. Additionally, TTPA exposures (1-100 nM), elicited a sex-dependent response in P-gp transport: increasing transport in males and decreasing transport in females. All TTPA exposures in transport were measured within a TTPA dose and time of 1-1000 nM and 1-6 hours respectively. Inhibitors of either transcription or translation abolished the TTPA dependent increases in male P-gp transport. Western blot and immunofluorescent assays confirmed the TTPA dependent P-gp expression increases in males and decreases in females. Inhibition of PPAR-y by GW9662 abolished the TTPA dependent increases in males but not the decreases in females. However, the TTPA decreases in female P-gp transport were blocked by the ER antagonist, ICI. This work indicates that environmentally relevant concentrations of TTPA (1-100 nM) alter ABC transporter function at the blood-brain barrier. Moreover, changes in BBB permeability alters brain homeostasis, modifies CNS drug delivery and increases brain exposure to harmful xenobiotic toxicants. This work was supported by the Intramural Research Program at NCI/NIEHS, ZIA BC 011476.
1336  Neurotoxic Effects of Erythrophleum ivorense in Experimental Animal Model

Erythrophleum ivorense (EI) is a common plant in tropical Africa. The stem bark of this plant is employed as an ordeal brew and causes a display of psychological symptoms, altered personality and several behavioral effects though the mechanism is yet to be understood. Sixty male Wistar rats were divided into five groups (A-E) of twelve rats each. Group A, control received distilled water. The test groups (B-E) were administered 10, 20, 30 and 40 mg/kg ethanol extract of EI in a single oral dose for 28 days. Cognition (Morris water maze, forced swim and tail suspension tests) and motor function (wire grip and inverted wire mesh grid grip tests) assessments were done. The brains were harvested and stained with Haematoxylin and Eosin (H&E), cresyl violet and immunohistochemistry was done using anti-Glial Fibrillary Acidic Protein (Z0334) (GFAP), anticalbin- din (D-28k), ionized calcium binding adapter molecule 1 (019-19741) (Iba-1) and Myelin Basic Protein (ab65988) (MBP) antibodies. At all tested doses, EI ivorense significantly (p<0.05) increased escape latency in the Morris water maze compared to control. A dose-related increase in duration of immobility was observed in the forced swim and tail suspension tests. There was also significant reduction in hanging latency in the wire grip and inverted wire mesh grid grip test. Depletion of cells in the Purkinje layer, CA1 and CA4 of the cerebellum and hippocampus was observed with H&E and cresyl violet. Immuno-staining revealed astrocystic activation in the cerebellum, loss of dendritic spines and arborization, microglial activation in the cerebrum, neuronal loss in CA3 and CA4 of the hippocampus, and demyelination in the cerebel- lum, corpus striatum and the dentate gyrus of the hippocampus. The ethanol extract of the stem bark of EI caused a dose-dependent deficit in learning, memory and motor coordination with evidences of depression in rats. It is concluded that the plant is neurotoxic and is able to induce several neurobehavio- ral changes in rats. Therefore, the use of this plant as ordeal poison should be reconsidered.

1337  Investigation of Protein Adduct Formation as Mechanism of Neurotoxicity in Rats Exposed to 1-Bromopropane

Following the frameworks of predictive toxicology, a recent US EPA report lists chemicals predicted to cause neurotoxicity based on the Hard Soft Acid Base (HSAB) Theory. Acrylamide, a soft electrophile which results in cumula- tive neurotoxicity through protein adduct formation, fits this theory based on experi- mental data. 1-bromopropane, a solvent widely used in adhesives, aerosols, and cleaning of metals and electronics, is predicted to behave similarly based on HSAB parameters (hardness, softness, electrophilicity). Due to widespread use, there is an increasing number of reported cases of neuropa- thy in workers exposed to 1-bromopropane; however, the mechanism driving the toxicity is not understood. We hypothesized that rats exposed to 1-bromo- propane would exhibit similar neuropathies compared to acrylamide, as a result of protein adduct formation in brain synaptosomes. To address this, we exposed rats orally to acrylamide (20 mg/kg) or 1-bromopropane (800 mg/ kg) via gavage. Gait was analyzed weekly or biweekly until a sufficient level of gait abnormality was achieved. Once euthanized, half of the rat brain was preserved to examine histological changes, while the other half was dissected for proteomic (synaptosomes) and gene expression analysis. Throughout the study, we observed decreased weight gain in chemical-exposed animals. Rats exposed to acrylamide began to show symptoms between weeks 4-5, while the neurotoxicity from 1-bromopropane did not present until 10-12 weeks. Unexpectedly, the symptoms of neurotoxicity were distinct between the two groups with acrylamide-exposed rats exhibiting foot splay, altered balance, and an inability to stand, while the 1-bromopropane rats exhibited an inability to right the hind legs and shuffling of hind feet. These contrasting symp- toms coincided with histological differences and diversity in protein adduct profiles generated by TMT proteomics. This study demonstrates the utility of chemical properties in predicting the mechanisms and toxicity of chemicals, as a way to better prioritize and regulate chemicals in the future.

1338  Dioxin induces Accumulation of Neurofilament Light Protein in Neurons: AHR-Mediated Transcriptional Upregulation through Enhancement of ERK1/2 MAPK Pathway
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It is known that exposure to and bioaccumulation of dioxins produce a wide variety of toxic and health effects, such as tumor promotion, teratogenicity, endocrine disruption and tissue- and organ-specific toxicities. The study of mechanisms of dioxins’ neurotoxicity is a hot area in the past years since signific- ant deficits in cognitive functioning have been reported in humans exposed to dioxins and dioxin-like compounds. Studies demonstrated that dioxin produced its neurotoxicities by disrupting neuronal differentiation and the function of neuro-transmission systems. In our studies, we found novel mech- anisms whereby dioxin may produce its biological or toxicological effects by decreasing neuronal AchE activity through a transcriptional down-reg- ulation mechanism via the AHR-dependent signaling pathway. Meanwhile, dioxin was also found to exert the disturbance on neuronal differentiation by up-regulating the expression of neurofilaments, the main component of neurites, also regarded as the biomarker for both neuronal differentiation and neurodegeneration. Furthermore, AHR mediates the transcriptional regula- tion of filament L through enhancement of the ERK1/2 MAPK pathway, which suggests a crosstalk between the two pathways in neurons.

1339  Proteomic Analysis Links Proteasome Inhibitors Induced Peripheral Neuropathy to Mitochondrial Toxicity in a Human Neuronal Cell Model
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Bortezomib (BTZ) is a boronic acid based and a reversible proteasome in- hibitor, whereas carfilzomib (CFZ) is an epoxymycin derivate and irreversible proteasome inhibitor. Both have been used successfully to treat haema- tological malignancies. However, neuropathic manifestations are dose limit- ing for BTZ as many other chemotherapeutic agents. CFZ is associated with lower incidence of neuropathy than BTZ by as yet unknown mechanisms. The aim of this study is investigating the effects of BTZ and CFZ on human neu- rons derived from human neural precursor cells for a better understanding of molecular mechanisms that cause different neurotoxic profiles. βIII-tubulin expression was validated by confocal microscopy to ensure neuronal differen- tiation. Neurotoxic effects of BTZ and CFZ on human neurons were anal- ysed by a proteomic approach using nano-HPLC/nano-ESI-LTQ-Orbitrap MS/ MS. Quantitative data from significant protein alterations were analysed by Pathway Studio Software v.11.4 to determine possible functional interactions. According to the results of the study, proteins regulating the mitochondrion organization and biogenesis, mitochondrion membrane permeability and respiratory chain, heat shock response, selective autophagy showed signifi- cant changes. Proteasome inhibition was measured by fluorometric assay and both drugs caused significant inhibition of proteasomal activity. BTZ caused mitochondrial membrane potential decrease where CFZ did not cause any change by flow cytometry. Mitochondrial mass per cell assessed by staining with Mitotracker Green FM and flow cytometry, showed significant reduction with BTZ treatment. LysoTracker Red and MitoTracker Green colocalisation under confocal microscopy showed that mitophagy was upregulated with BTZ treatments. BTZ caused higher upregulations in the protein levels of heat shock response (HSP) HSP 32, HSP 60, HSP 70, HSP 90 and autophagy receptor protein p62 than CFZ. According to our study results, the higher neurotoxic pro- file of BTZ may be related to more severe mitochondrial toxicity, heat shock response and selective autophagy in neurons. This study supported by TUBITAK, Grant Number: 216S838.

1340  3D Neurone-on-a-Chip Model Effectively Screens Neurotoxic Compounds
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Organ-on-a-chip devices that mimic in vivo physiology have the potential to identify chemical and drug toxicities in earlier preclinical stages of develop- ment while relying less heavily on animal models. We have developed a nerve-on-a-chip construct to culture animal and human neural tissue in a dual hydrogel system that promotes axon growth analogous to mature nerve
anatomy. Here we culture rat sensory dorsal root ganglia (DRG) in the
construct to demonstrate its potential as a preclinical assay for screening impli-
cations of nerve dysfunction by measuring electrical signals in tissue exposed to
4 chemical drugs known to cause peripheral neuropathy. The dual hydrogel construct consists of a cell-impermeable polyethylene glycol (PEG)
outer layer and a growth-permissive inner gel layer that permits bundled nerve-like growth. DRG tissue from E15 rat pups was inserted into the inner
gel and cultured for 4 weeks until mature. The constructs were exposed to Bortezomib, Oxaliplatin, Paclitaxel, or Vincristine, all common chemothera-
peutics for various cancers, at doses ranging from 0.1 to 1.0 μM. After 7 days of treatment, compound action potentials (CAP) were measured by electric-
ally stimulating the capsaicin-sensitive Aδ fibers. All nerve cultures had a 500 mg/kg group in the

DMAB body. We collected nerve conduction velocity (NCV) and the peak amplitude (AMP), which are two electrophysiological clinical metrics indica-
tives of healthy or diseased populations. A trend was observed towards de-
creased NCV and AMP in a dose-dependent manner across all drugs. At high
drug concentrations, NCV and AMP measurements were typically lower than Control by 10-60%. Fluorescent microscopy revealed that the robust growth of
axons remained intact following drug exposure. Only in the high doses there was a visible loss of axon density. Maintained cell viability in low doses was
confirmed with CCK-8 and LDH viability assays. Dose-response curves of evoked
neurons displayed that significant functional pathologies occur before
important changes in cell viability as measured in the CCK-8 assay. This indi-
cates that functional, clinically-relevant measurements represent a more sensi-
tive metric. Our data suggests electrophysiology recordings collected from our
nerve-on-a-chip platform can closely track subtle pathologic changes in nerve function, demonstrating the feasibility of using this in vitro nerve-on-a-
chip model as a preclinical screen for peripheral neuropathy.

1341 The Metabolomic Profile of Monoaminergic Neuronal Perturbation in Caenorhabditis elegans

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Neurodegeneration is a complex phenomenon that has environmental, ge-
etic, and aging risk factors. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropy-
ridine) is known to induce Parkinsonism in humans and animal models. Its active metabolite MPP+ (1-methyl-4-phenylpyridinium), is a complex I inhibi-
tor that weakly binds to the mitochondrial carrier of mono-
aminergic neurons, the vesicular monoamine transporter 2 (VMAT2; SLC18A2)
transports monoamines into vesicles, where they reside until release into the
synapse, protected from cytoplasmic degradation. VMAT2 can also sequester
MPP+, preventing it from exerting its toxicity on the mitochondria. Altered
expression of VMAT2 has been associated with susceptibility to MPP+ and
Parkinson’s disease, as observed in both animal and human studies. To mea-
sure holistic changes in biochemical responses from genetic mutations and
chemical exposures in C. elegans, we have developed a high-resolution me-
tabolomics framework using a Thermo QExactive LC Orbitrap that leverages the
sensitivity of high-resolution mass spectrometers and the unparalleled genetic
tracability of the nematode model. In studies using both a mutant strain lacking the VMAT2 orthologue cat-1 and the potent neurotoxicant
MPP+, we detected changes in features suggestive of altered monoamine
metabolism. Differentially expressed metabolic features were evaluated using metabolic pathway enrichment analysis, which detected altered fatty acid β-oxidation,
which is known to be regulated by the monoamine serotonin in C. ele-
gans. In wildtype L4 worms exposed to 1 μM MPP+ for 4 hours, we detected
changes in tryptophan metabolism and altered levels of 5-hydroxyindole ac-
eate, the primary metabolite of serotonin, suggesting additional changes in
serotonin pathways. Wildtype worms in the first larval stage exposed to 2μM
MPP+ for 4 hours show an increase in neuronal blebbing (p < 0.0001) compared to controls. Using fluorescent markers of neuronal integrity and
metabolomics, we can identify genetic and environmental contributors to
neurodegeneration and compare metabolomic profiles to elucidate underly-
ing biochemical pathways of neurodegeneration.

1342 14-Day Dermal Toxicity Study in B6C3F1/N Mice and HSD:Sprague-Dawley Rats Exposed to Dimethylamine Borane (DMAB)

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Dimethylamine borane (DMAB) is a reducing agent used in the manufacturing
of electronics and other industrial processes. DMAB is a known irritant to
the eyes, skin, and respiratory tract, and one incident involving occupational

...exposure provides some evidence to suggest that DMAB may be a neurotox-
icant. Given the limited toxicity data available and the potential for occupa-
tional exposure, DMAB was nominated to the National Toxicology Program to
evaluate potential systemic and neurotoxic effects following dermal ex-
posure. In this study, male and female B6C3F1/N mice and Harlan Sprague
Dawley (HSD) rats (n=5/sex/species) were exposed to vehicle (95% ethanol), 15.6, 62.5, 125, 250, or 500 (B6C3F1/N female only) mg DMAB/kg body weight via dermal administration for 14 days.

Animals were assessed for clinical signs of toxicity, histopathological
lesions, and alterations in neurobehavioral function (fore- and hindlimb
grip compression strength and footspan distance). All male mice survived to
study completion and treatment-related effects were limited to clinical observa-
tions of nasal and/or ocular discharge in all males at doses ≥ 125 mg/kg and in
two females administered 250 mg/kg DMAB. Plasma concentrations of DMAB,
dimethylamine (DMA), and N-nitrosodimethylamine (NDMA), however, were
not detected above the limit of quantitation in males at 6 hours (0 and 125 mg/kg B6C3F1/N; 0 and 62.5 mg/kg HSD) or in males and females from all dose
groups 24 hours following the last application. Overall, these data sug-
gest that DMAB is overtly toxic to female B6C3F1/N mice at levels ≥ 250 mg/
kg, and B6C3F1/N mice are more sensitive to dermal exposure to DMAB than
the HSD rat. While this preliminary study provides evidence to suggest that
DMAB may be neurotoxic in B6C3F1/N mice, additional studies are needed to
fully characterize the neurotoxic potential of DMAB in mice.

1343 Chronic, Low-Level Oral Exposure to Marine Toxin, Domoic Acid, Alters Whole Brain Morphometry in Nonhuman Primates


Domoic acid (DA) is an excitatory neurotoxin produced by marine algae and
responsible for Amnesiac Shellfish Poisoning in humans. Current regulatory
limits (approximately 0.075-0.1 mg/kg/day) protect against acute toxicity, but
recent studies suggested chronic ingestion of DA resulted in neurotoxicity near the current human regulatory limit are related to structural and chemical
changes in the brain.
results show that 4-OH PCB 52 is more toxic than PCB 52 to N27 cells - a dopaminergic cell line. Our work intends to understand how PCB 52 and its metabolites modulate the dopaminergic system. This includes analyzing dopamine metabolism in cell culture and rat brain tissue. We have also performed preliminary studies on detecting reactive oxygen species. Furthermore, we will analyze changes in gene expression to explore alterations in dopamine cell trafficking. PCB 52 and 4-OH PCB 52 have previously shown toxicity in neurogenic cell lines. However, not much is known about how these compounds alter and modulate the dopaminergic system. Future studies will employ neuroprotective strategies.

### 1345 Dietary Strategies Affect Marine Algal Toxic Levels in Subsistence Harvested Alaskan Pinnipeds

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Domoic acid (DA) and saxitoxin (STX) are marine algal-produced toxins that elicit acute neurotoxic symptoms in mammals following ingestion. Trophic transfer of both toxins allows for their spread through marine food webs. Cases of acute DA and STX toxicity are termed Amnesiac Shellfish Poisoning and Paralytic Shellfish Poisoning respectively, as the primary vector of human exposure is contaminated shellfish. Knowledge of these toxicoses has led to the implementation and enforcement of seafood safety regulatory limits for both DA and STX in shellfish (20 and 0.8 ppm, respectively). However, human exposure to algal toxins can occur through additional vectors. Native communities in Alaska conduct annual subsistence harvests of marine mammals that are a part of complex marine food webs. As DA and STX prevalence in Alaskan Arctic food webs has not been extensively quantified, such harvests are of interest both with regard to potential human toxic exposure and to marine mammal health. We tested 856 samples collected from the gastrointestinal tracts of pinniped subsistence harvest in Alaska between 2003 and 2016. Samples were analyzed for DA and STX presence using commercially available Enzyme-linked Immunosorbent Assay (ELISA) kits. Toxin prevalence was found to be highest in bearded seals and walruses and lowest in ribbon seals. Maximum DA and STX concentrations followed similar trends. Additionally, few samples were found to have toxin concentrations above the seafood safety limits for acute toxicity. Dietary strategy is suggested as one explanation for interspecies variations in toxin level, though the clarity of this relationship is complicated by features of the opportunistic dataset as well as oceanographic, seasonal, and physiological factors.

### 1346 Behavioral and Histological Evidence of a Neuroimmune Basis for Gulf War Illness

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Chronic exposure to the gluccorticoid, corticosterone (CORT), at levels associated with high physiological stress, has been shown to prime the neuroimmune response to neurotoxic exposures and systemic inflammation, significantly increasing the expression of proinflammatory cytokines/chemokines following exposure. Gulf War Illness (GWI) is a multi-symptom, neuroimmune-based disorder that presents with features characteristic of persistent sickness behavior. Using a preclinical mouse model of GWI, we have found that chronic exposure to the stress hormone corticosterone (CORT; 200-400 mg/L) in the drinking water for 7 days exacerbated the initial neuroinflammatory response to the sarin surrogate diisopropylfluorophosphate (DFP; 4 mg/kg, i.p.). A more recent study using this exposure protocol has found that CORT+DFP exposed animals exhibit cognitive impairment in the Novel Object Recognition Test with decreased discrimination of the novel versus familiar object. However, this acute exposure model is more representative of the veterans’ time in theater, and those suffering with GWI are nearly 30 years removed from their tours of duty. Thus, a more extended duration animal model is necessary. Our model of GWI at 5 weeks after the initial CORT+DFP event, constituting periodic administration of CORT for 7 days every other week and a subsequent systemic immune challenge with the bacterial mimic lipopolysaccharide (LPS; 0.25-0.50 mg/kg, s.c.) mimics the long-term illness and ‘flare up’ of symptoms as is reported in GWI. This longer CORT regimen produced signs of decreased cognition in the Novel Object Location Test, signified by CORT+DFP+LPS animals less able to distinguish the displaced versus familiar object. This paradigm reveals a further exacerbation of neuroinflammatory markers and emergence of activated microglia in key brain areas including hippocampus and cortex that affects behavior for at least 12 days after the last LPS dose. Together, these data provide additional support that GWI is a chronic, stressor-primed, neuroinflammatory condition with adverse long-term neurobiological and behavioral outcomes. Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention.

### 1347 Comparison of Acute Effects of Neurotoxic Compounds on Network Activity in Human and Rodent Neural Cultures

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Assessment of neuroactive effects of chemicals in cell-based assays remains challenging as complex functional tissue is required for biologically relevant readouts. Recent in vitro models using rodent primary neural cultures grown on multimicroelectrode arrays (MEAs) allow quantitative measurements of neural network activity and have been demonstrated to be suitable for neurotoxicity screening. However, robust systems for testing effects on network function in human neural networks are still lacking. The increasing number of differentiation protocols for generating neurons from induced pluripotent stem cells (iPSCs) holds great potential to overcome the unavailability of human primary tissue and expedite human cell-based assays. Yet, the variability in neuronal activity, prolonged ontogeny and rather immature stage of most neuronal cells derived by standard differentiation techniques greatly limit their utility for screening neurotoxic effects on neuronal networks. Here, we used excitatory and inhibitory neurons that were separately generated by direct conversion from human iPSCs together with primary human astroglial cells to establish high-fidelity microBrain models. 3D-grown neuron/glia co-cultures showed pronounced neuronal activity and robust formation of synchronized network activity on MEAs, albeit with noticeable delay vs primary rat cortical cultures. We further investigated the effects of neurotoxic test compounds, including 4 GABA receptor antagonists, an organotin, and 4 pyrethroid insecticides, as well as 3 negative control compounds on network activity (in these human neuron/glia co-cultures). Importantly, we observed largely corresponding dose-dependent alterations in firing, burst and synchrony metrics of neuronal network activity in iPSC-derived human neuron/glia and rat primary cortical cultures. These results demonstrate the utility of this direct-differentiated human model for neurotoxicity screening using MEAs.

### 1348 Validation of High-Throughput 3D microBrain Model to Predict Drug-Induced Neurotoxicity across a Diverse Set of Pharmaceuticals


Drug-induced central nervous system (CNS) toxicity is a top three cause of safety-related attrition across the pharmaceutical industry. Undesired side-effect on CNS in human account for 10% of all drugs withdrawn from sale during the periods 1960-1999. A main reason for the high failure rate is that the concordance rate is low between human adverse drug reaction (ADR) and identification in preclinical toxicity studies. The development of predictive CNS toxicity assays is needed to help pharmaceutical companies design and optimize safer therapies. Over a dozen different IPS-derived microBrain and miniBrain models have been published in recent years, but their ability to predict CNS toxicity in patients is as of yet unproven. To evaluate the predictive capabilities of one of these models, we validated an IPS-derived microBrain model using 84 structurally diverse pharmaceuticals, a combination of US FDA-approved drugs and clinical drug candidates with varying levels of seizurogenic and neurodegenerative liability. Seven endpoints were analyzed using Ca oscillations and cellular ATP levels after a single and repeated exposure treatment, respectively. The microBrain in vitro IC50 for each endpoint were rooted into clinical used therapeutic exposure (fCmax) as classifiers (ICmax/IC50) to predict clinical drug-induced seizures and neurodegeneration. We used logistic regression with clinical reported CNS toxicity as binary response variable and combined each endpoint as independent variables (ICmax/IC50) to calculate probability for CNS toxicity. After refining these endpoint cutoffs on separate training and test sets, we find that in total...
the human microBrain gave us a specificity 93.33% and sensitivity 53.49%. Importantly, this high throughput model has very low false positive rate in the prediction of seizures, convulsions and neurodegeneration. This assay has the potential to be used as a predictive assay during early drug discovery phase for neurotoxic hazard identification. It can be incorporate readily into the pharmaceutical toxicological screening paradigm, aiding the early identification of compounds that eventually may fail due to CNS toxicity.

1349 Anatomical Features of Brain Slices That May Be a Source of Variability When Measuring the Effect a Compound Has on Synaptic Plasticity


Examining field potentials evoked from acutely prepared slices of rodent brain tissue (particularly from the hippocampal area) offers an excellent means for preliminary screening of neurotoxicity. Whereas many studies using this approach will measure effects on basal synaptic transmission alone, studying synaptic plasticity, as well, can provide additional important information on a compound’s neurotoxicity. Of the various forms of synaptic plasticity available for study, long-term potentiation (LTP) has received significant attention, given its presumed role as a critical cellular mechanism underlying learning and memory. However, variation in LTP expression and magnitude is often seen across studies, and may serve to discourage some from using the measure in their work. Notably, some of the variability may arise (at least, in part) because many studies using LTP ignore: 1) the possibility that tissue harvested from the two poles of the hippocampus (i.e., septal and temporal) may not respond uniformly; and 2) the precise site of response recording, given that not all synapses within a brain region are homogenous. Hence, using slices prepared from the hippocampal region of adult-electrode array recording system, we investigated the difference in LTP induced by electrical stimulation (tetanus, 2 x 100 Hz) as a function of both distance between the cell body layer and the recording site (100, 200, 300, 400 µm away, within the CA1 sub-field), and the pole from which the slices were harvested (septal, or temporal). Preliminary results show that LTP expression is different among the four recording points and between poles. Specifically, tetanus-induced the greatest magnitude of LTP expression at points that were 100 µm from the soma; furthermore, potentiation in temporal slices was found to be the greatest. In addition, slices from the temporal pole took longer to reach peak post-tetanus potentiation relative to slices from the septal pole. Our observations reveal that LTP expression within hippocampal slices can be affected both by the distance of the recording site from the soma and where along the longitudinal axis the slice was found. As a result, we have demonstrated the need to consider both the relative recording position and the anatomical pole of origin when measuring LTP in acute hippocampal slices.

1350 An Engineered 3D Peripheral Human “Nerve-on-a-Chip”: A Novel Assessment for Neurotoxicity In Vitro

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The development of human iPSC-derived neurons has greatly expanded the predictive potential of 2D preclinical assays. Additionally, these iPSC-derived neurons are more accessible than neural stem cells for tissue engineering and screening purposes. Concurrently, the use of engineered microphysiological systems (MPSs), Organs-on-Chips, and 3D organoids have seen tremendous growth as a preclinical drug screening tool because they are more biometric than 2D assays. Especially when trying to model the complexity of the human nervous system, 3D engineered cultures provide marked advantages by recapitulating cell-cell interactions. However, a limited focus has been given to MPSs that mimic peripheral nerves (PNs) even though peripheral neuropathy is implicated in numerous disease states and is a common and significant side effect of many therapies. In this study, we fabricated a novel in vitro human-iPSC based 3D nerve that supports axon growth analogous to PN anatomy. This in vitro nerve can provide clinically relevant metrics such as nerve conduction velocity (NCV) and histological ultrastructure. These read-outs represent the gold-standard in preclinical testing and, previously, were only obtainable through in vivo experimentation. Using low adhesion microplates, self-assembling spheroids comprised of either iPSC-derived human nerves alone or co-cultures with primary human Schwann cells and iPSC-derived human nerves were fabricated. Over 4 weeks, the nerves were grown in a 3D environment to reach maximum lengths of 5 mm, with diameters varying from 0.015% to 0.001%. Interestingly, two AFFF did not affect cellular viability at any concentration, whereas many studies using this approach will measure effects on basal synaptic transmission alone, studying synaptic plasticity, as well, can provide additional important information on a compound’s neurotoxicity. Of the various forms of synaptic plasticity available for study, long-term potentiation (LTP) has received significant attention, given its presumed role as a critical cellular mechanism underlying learning and memory. However, variation in LTP expression and magnitude is often seen across studies, and may serve to discourage some from using the measure in their work. Notably, some of the variability may arise (at least, in part) because many studies using LTP ignore: 1) the possibility that tissue harvested from the two poles of the hippocampus (i.e., septal and temporal) may not respond uniformly; and 2) the precise site of response recording, given that not all synapses within a brain region are homogenous. Hence, using slices prepared from the hippocampal region of adult-electrode array recording system, we investigated the difference in LTP induced by electrical stimulation (tetanus, 2 x 100 Hz) as a function of both distance between the cell body layer and the recording site (100, 200, 300, 400 µm away, within the CA1 sub-field), and the pole from which the slices were harvested (septal, or temporal). Preliminary results show that LTP expression is different among the four recording points and between poles. Specifically, tetanus-induced the greatest magnitude of LTP expression at points that were 100 µm from the soma; furthermore, potentiation in temporal slices was found to be the greatest. In addition, slices from the temporal pole took longer to reach peak post-tetanus potentiation relative to slices from the septal pole. Our observations reveal that LTP expression within hippocampal slices can be affected both by the distance of the recording site from the soma and where along the longitudinal axis the slice was found. As a result, we have demonstrated the need to consider both the relative recording position and the anatomical pole of origin when measuring LTP in acute hippocampal slices.

1351 Effects of 10 New Generation PFAS–Containing Aqueous Film Forming Foams (AFFF) on Human Liver Cell Viability

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Per- and polyfluoroalkyl substances (PFAS) are man-made chemicals characterized by their carbon-fluorine bonds, and resistance to heat, water, and oil. PFAS have been used in hundreds of industrial applications and consumer products, including aqueous film forming foams (AFFF), and as such have contaminated human food and water sources. The purpose of this study was to evaluate the cytotoxicity of 10 AFFFs, thought to be in current use in 2018, in a human hepatocyte/cholangiocyte cell line (HepaRG). In previous work, we have demonstrated that AFFF are particularly sensitive to many adverse effects of PFAS, so confluent HepaRG cells were exposed to the AFFF by blinded allocation for 72 hours at a concentration range of 0.001% to 2% in media. CellTiter-Glo luminescence assay detected substantial declines in cellular viability at concentrations between 0.25% and 0.5%, and total cell death at concentrations of 1% and 2% for several AFFF. Three AFFF produced significant cell death at concentrations as low as 0.01%, with an EC50 as low as 0.015%. Interestingly, two AFFF did not affect cellular viability at any concentration tested. However, noticeable dose-dependent morphological changes were evident in the cell cultures that were exposed to one of those two AFFF. Accumulation of lipid droplets were seen in hepatocytes, specifically. Unsupervised high-resolution mass spectrometry analysis of the AFFF detected no 8-carbon PFAS, but numerous 6-carbon PFAS were present, many of which were similar in mass. Further studies will identify hepatocyte accumulations, and the active fraction of the AFFF. The AFFF with the highest EC50 and least biological toxicities will be prioritized for testing in vivo, in hopes of identifying AFFF with little/no toxicities.

1351a Different Human-Induced Pluripotent Stem Cell Models for In Vitro Neurotoxicity Assessment

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Neurotoxicity testing largely relies on time consuming and ethically debated animal experiments that are neither amenable for high-throughput screening nor fully predictive for human risk. Nowadays, there is an increasing availability of human induced pluripotent stem cells (hiPSC) from varying donors differentiated in different neuronal cell types such as glutamatergic, GABAergic and dopaminergic neurons as well as astrocytes. Such hiPSC models can circumvent interspecies translation and thus hold great promise for replacing the current gold standard of (rodent) primary cultures and future neurotoxicity testing. The present study therefore aims at investigating the usability of different (commercially available) hiPSC-derived neuronal models for in vitro neurotoxicity screening. We cultured different hiPSC-derived neuronal models with different ratios of GABAergic and glutamatergic neurons in the presence of astrocytes. Using immunofluorescent stainings, we confirm the mixed neuronal nature of these hiPSC-derived neuronal co-cultures. Furthermore, using multiwell micro-electrode arrays (mwMEA), we demonstrate that these hiPSC-derived co-cultures can develop spontaneous neuronal network activity and (network) bursting behavior. As a proof of concept for neurotoxicity testing, we exposed our cultures to the seizurogenic compounds picrotoxin (PTX), 4-aminopyridine (4-AP) and strychnine. Our data indicate that spontaneous neuronal activity and (network) bursting are concentration-dependently modulated by these compounds. While further characterization and validation is required to facilitate the acceptance of these physiologically-relevant hiPSC-derived neuronal co-cultures for neurotoxicity testing, our data indicate that these models represent the cellular heterogeneity of the in vivo brain, develop spontaneous activity and are amenable for in vitro neurotoxicity and seizure liability testing. This work was funded by NCI3Rs (project number 50308-37216), ZonMW (project number 11407201) and the Faculty of Veterinary Medicine (Utrecht University, Utrecht, The Netherlands).
1352 iPSC Derived Human 3D Brain Model to Study Developmental Neurotoxicity Adverse Outcome Pathways (DNT AOP)

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A variety of molecular and cellular processes is known to be crucial to proper development and function of the central nervous systems (CNS), making this stage one of the most sensitive exposure windows for chemical insult. Current regulatory test strategies are high cost and long. Moreover, systematic testing of DNT is not a standard requirement in chemical legislation safety assessment. In consequence, there is a critical lack of knowledge when it comes to toxicity of drugs and other xenobiotic chemicals on the developing brain. This together with the increase of developmental disorders such as autism and hyperactivity syndromes have increase the awareness that we could be facing a silent pandemic. An AOP represents the existing knowledge concerning the causal links between the molecular initiating event and the cascade of key events (KE) that lead to a specific adverse outcome of regulatory concern. Adverse outcome pathways (AOPs) are expected to guide identification of experimental testing and non-testing approaches to support regulatory decision-making referring also to developmental neurotoxicity evaluation. Recently an AOP13 has been endorsed by OECD, describing how inhibition of glutamatergic spine growth is associated with hyperactivity in humans and zebrafish. Our goal is to determine how AhR activation alters zebrafish brain development and function due to impaired expression of the transcriptional changes that lead to developmental defects. Epidemiology concerns the causal links between the molecular initiating event and the environment relevant compounds with various anticipated MoAs. We found that exposure-induced behavioral alterations were reproducible and dependent on concentration and time. Comparative and quantitative analyses of the obtained locomotor patterns revealed that behavioral effects were not restricted to compounds primarily known to target the nervous system. A clear distinction of MoAs based on locomotor patterns was not possible for most compounds. Furthermore, chemicals with an anticipated same MoA did not necessarily provoke similar behavioral phenotypes. Finally, we determined an increased sensitivity (≥10-fold) compared to observed mortality in the LMR assay for 5 of 8 neuroactive chemicals as opposed to non-neuroactive compounds. This study (Leuthold et al.) is currently under review in ES&T.

1354 Can Environmentally Relevant Neuroactive Chemicals Specifically Be Detected with the Locomotor Response Test in Zebrafish Embryos?

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Chemicals considered as neuroactive (such as certain pesticides, pharmaceuticals and industrial chemicals) are among the largest groups of bioactive substances recently detected in European rivers. However, the determination of nervous system-specific effects has been limited using in vitro tests or conventional endpoints including lethality. Thus, neurobehavioral tests using in vivo models (e.g. zebrafish embryo) have been proposed as complementary approaches. To investigate the specificity and sensitivity of a light-dark transition locomotor response (LMR) test in 4 to 5 days post fertilization zebrafish with respect to different modes of action (MoAs), we analyzed a set of 18 environmentally relevant compounds with various anticipated MoAs. We found that exposure-induced behavioral alterations were reproducible and dependent on concentration and time. Comparative and quantitative analyses of the obtained locomotor patterns revealed that behavioral effects were not restricted to compounds primarily known to target the nervous system. A clear distinction of MoAs based on locomotor patterns was not possible for most compounds. Furthermore, chemicals with an anticipated same MoA did not necessarily provoke similar behavioral phenotypes. Finally, we determined an increased sensitivity (≥10-fold) compared to observed mortality in the LMR assay for 5 of 8 neuroactive chemicals as opposed to non-neuroactive compounds. This study (Leuthold et al.) is currently under review in ES&T.

1355 Evaluating the Developmental Toxicity of Halogenated Pyrroles in Zebrafish

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Halogenated pyrroles, or halopyrroles, are one of the largest classes of halogenated alkanoids. Anthropogenic halopyrroles are increasingly detected in wastewater and drinking water. While they are currently an unregulated class of disinfection by-products, halopyrroles are generating public health concern due to their persistence in the environment, and recent findings indicating that these compounds are cytotoxic and interfere with development in worms. We recently demonstrated that anthropogenic halopyrroles dysregulate calcium dynamics in microsomes isolated from rabbit fast-twitch skeletal muscle by sensitizing ryanodine receptors (RyRs). Because of the importance of calcium signaling in vertebrate development, these observations raise the question of whether halopyrroles interfere with development in vertebrates. To address this question, teratogenic effects and photomotor response were assessed in developing wild-type (Tropical 5D) zebrafish (Danio rerio) exposed to varying concentrations (0.03 μM to 30 μM) of three different halopyrroles: tetrabromopyrrole, 2,3-dibromomaleimide, and 2,3-dibromo-N-methylmaleimide. Zebrafish embryos were dechorionated and exposed via static waterborne exposure to halopyrroles beginning at 6 h post-fertilization through 5 days post-fertilization (dpf). Zebrafish were observed daily for gross teratological malformations and mortality. Behavioral tests were conducted at 4 and 5 dpf using the Noldus automated tracking system to assess effects of the compounds on an apical endpoint of developmental neurotoxicity. Tetram bromopyrrole and 2,3-dibromo-N-methylmaleimide were embryonic lethal at 1 μM and 30 μM, respectively, while 2,3-dibromo-N-methylmaleimide was not lethal at any concentration tested. Developmental malformations were only observed in fish exposed to tetram bromopyrrole at 0.3 μM to 1 μM. Photomotor response was significantly altered only by 2,3-dibromo-N-methylmaleimide which decreased swimming during dark phase in a non-monotonic concentration-response related manner. Given the potential for human exposure to anthropogenic halopyrroles, these observations suggest that further evaluation of the developmental neurotoxicity of this class of compounds in vertebrate species is warranted. Supported by the NIEHS (R01 ES014901, PBL & INP).

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1356 Identifying Neurophysiological Signatures of Neurotoxic Action Using Classification Models

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Exposure to environmental chemicals during nervous system development can result in developmental neurotoxicity (DNT). In vitro microelectrode array (MEA) recordings of the ontogeny of neural network function following chemical exposure can be used to screen chemicals for DNT hazard. These recordings capture temporal and spatial aspects of action potential activity, which are described by a set of network parameters. To date, ~250 compounds have been tested in this assay, but with the same molecular initiating event (MIE) have similar effects on network parameters that have not been explored. This could lead to identification of “fingerprints” for DNT compounds that provide information useful for determining the MIEs for unknown compounds. Here, effects of 45 previously tested compounds with varying modes of action were studied by comparing neural network recordings of 16 voltage-gated sodium channel modulators, 11 GABA agonists and antagonists, and 18 acetylcholinesterase inhibitors. These data were used to investigate the relationship between neural network recording parameters and the mode of action of the compounds. This approach could lead to a better understanding of the mechanisms underlying DNT and the ability to identify compounds with similar modes of action.

1358 Zebrafish Larvae Require Specific Strains of Bacteria to Allow for Control-Like Neurobehavioral Development

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There is an increasing appreciation of the relationship between gut microbiota and neurodevelopmental function. We previously showed that axenic (microbe-free) zebrafish are hyperactive at 10 days post fertilization (dpf) relative to colonized zebrafish. Interestingly, while exposure to heat-killed bacteria or microbe-associated molecular patterns failed to block hyperactivity in axenic larvae, colonization of axenic zebrafish with Vibrio cholerae produced locomotor activity similar to colonized controls. These data suggest that there is a developmental requirement for microbial colonization to modulate host behavior. To address this hypothesis, nineteen bacterial isolates were obtained from 10 dpf conventionally colonized zebrafish. 16S rRNA gene sequencing identified five unique gram-negative isolates: Acinetobacter, Vibrio, Comamonas, Comamonadaceae, and Aeromonas. Monoclonization of axenic embryos at 1 dpf with 100 cells/mL of Acinetobacter, Comamonas, or Comamonadaceae resulted in behavioral profiles that partially blocked axenic-related hyperactivity. In comparison, axenic embryos monoclonalized at 1 dpf with Vibrio, Comamonas, or Aeromonas also blocked hyperactivity. These data suggest that specific bacterial taxa are sufficient for control-like neurobehavioral development while colonization with other strains of bacteria may influence motor behavior. These findings raise the possibility that environmental chemicals may disrupt neurobehavioral development by selecting for specific classes of host-associated microbes. This abstract does not represent US EPA policy.

1357 Thyroid Toxics and Neurodevelopment: Molecular Initiating Event May Be an Important Consideration

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Developmental neurotoxicity is a primary concern for thyroid disrupting chemicals (TDCs). Many environmental TDCs include antimicrobials, pesticides, flame retardants, and perfluorinated compounds. These TDCs reduce circulating levels of thyroid hormones (THs) by direct and/or overlapping mechanisms, many of which are not well characterized. It is unclear if these chemicals also reduce THs in the brain and cause neurodevelopmental deficits. This study investigated the potential developmental neurotoxicity of 14 of these chemicals, and demonstrated a decrease in THs in the brain but not circulating THs. Pregnant rats were dosed with a single high dose of triclosan (300mg/kg/day) or gavage or vehicle control once daily from gestational day 6 (GD6) to postnatal day 21 (PN21). Results show that triclosan did not induce changes in litter size or pup body weight. No effects were seen on liver weight or body-weight ratios in dams or pups, but liver metabolism genes were increased in expression. Serum total and free T4 were reduced in the dam (GD20 and PN21) and pup (PN0, PN2, PN6, PN14) to varying degrees; however, thyroid stimulating hormone (TSH) was unchanged. In the neonatal brain, no gene expression changes associated with TH dysfunction were detected, and there was no evidence of a TH-dependent phenotype (hypothyrotoxia). Neither were behavioral tests of learning and memory (trace fear conditioning) or sensory motor function (prepulse inhibition) impaired in adult offspring. These data suggest that despite reductions in serum T4 in dams and offspring, according to these metrics, the developing brain does not appear to be adversely affected. Further study regarding the relationship between THs in the brain and more sensitive measures of neurodevelopmental impairment are needed to more fully characterize the risk of chemicals like triclosan and others with similar mode(s) of action. Disclaimer: This work does not reflect EPA policy.

1359 Cytotoxicity of Quantum Dots on Human Neural Progenitor Cells Are Influenced by Their Surface Chemistry and the Sex Origin of Cells

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Quantum dots (QDs) are highly promising nanomaterials for clinical imaging/diagnosis due to their advantageous optical properties. They are a diverse class of nanostructures, varying in their composition (core, surface chemistry) and design (shape, shell thickness). However, safety is the major barrier in translating QDs to clinical uses. Brain development is an extremely intricate process, and its disruption may have severe and long-lasting consequences on brain structure/function. Brain function can be differentially affected by chemicals due to sex-specific regulatory mechanisms of brain development. We evaluated the cytotoxicity potential (LDH assay) of two CdSe/ZnS QDs with different surface chemistries (carboxyl functional group - ITK1, polyethylene glycol (PEG) without reactive functional group - Qtracker1), on proliferating and differentiating human neural progenitor cells (hNPCs). Sex-specific cytotoxicity of QDs was evaluated by the use of hNPC lines derived from males (NSC-H14) and females (hNPC1). Cadmium chloride (Cd) was tested under the same conditions in order to determine if the cytotoxicity mechanism of QDs is related to ion release. Exposures (days in vitro 1, 24 hours) to ITK1 at five different doses (2.5-40 nm) demonstrated a significant dose-dependent decrease in viability of both cell lines (NSC-H14, hNPC1) during the proliferation stage. Moreover, NSC-H14 (male) were more susceptible to the cytotoxicity of ITK1 than hNPC1 (female). Qtracker and Cd, at the same dose range as ITK1, did not induce cytotoxicity either in the proliferation and differentiation stages of either hNPC line. Our results show that surface coating defines the cytotoxicity of QDs on hNPCs and suggests that PEGylation can be a strategy for the safe use of QDs on clinical settings. In addition, our data supported the novel use of hNPCs from male and female origin as an in vitro model for examining sex-specific impacts of chemicals on brain development. This project is supported by US EPA (RD 83573801, RD 83541001) and the NIEHS (SP01ES009601, SP03ES00703, T32ES015453). The views expressed in this paper are those of the authors and do not necessarily reflect the views of the US EPA.
**1360 Neurodevelopmental Effects of a Non-Dioxin-Like Polychlorinated Biphenyl (PCB153)**


Polychlorinated biphenyls (PCBs) are persistent legacy contaminants that bioconcentrate in fish and other predators. Fish are a major dietary source of exposure for humans. One of the most abundant PCBs found in aquatic biota and in human maternal plasma and amniotic fluid is the planar, non-dioxin like PCB153, yet its biological activity is not well understood. There is increasing evidence that PCB153 targets the nervous system and may be responsible for developmental delays following exposures in early development. In this study, we used the zebrafish larvae to investigate the effects of PCB153 on the neural circuitry during development. We tested behavioral effects in PCB treated fish by assessing the larval escape response, and several other behaviors such as locomotion and preference for light or dark. Our results show that PCB153 dramatically increased the latency and maximum head turning angle in the escape response in 6-day old larvae. The escape response is driven by a neuronal circuit involving detection of the signal in auditory hair cells and other sensory neurons, and transmission to the Mauthner cells and other reticulospinal neurons. By using immunohistochemistry and electrical stimulation, which bypasses the sensory system and directly activates the Mauthner neurons, our data indicate that these neurons are present and functional. PCB153 is likely disrupting the function of the neural circuit in the hindbrain between the sensory ganglia and the Mauthner cells leading to increased latency and turning angle. Mutations in the axon guidance receptor (dcc) as well as a transporter regulating glutamate levels in synaptic regions (slc1a2b) phenotype the observed increased turning angle. Whether pathways involved in these genes are affected by PCB153 is not known. Targeted transcriptomics of brain from PCB153 exposed larvae compared to brain from control larvae is under way. The zebrafish model provides insight into both the ecological relevance of rapid escape from predators, and the human health relevance of proper neuronal development. This study will advance our understanding on the mechanism of action of an ubiquitous environmental contaminant. NIH 5P42ES007381 - The Boston University Superfund Research Program.

**1362 The Role of ATP13A1 in the Developing Brain: Effects on Locomotor Activity and Motor Function**

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ATP13A1 and ATP13A2 are P-type transport ATPases. ATP13A2 mutations result in a rare form of Parkinson’s disease and increased susceptibility to manganese exposure. ATP13A1 plays an important role in the normal functioning of the endoplasmic reticulum and is widely expressed during brain development. A previous human study identified a mutation in ATP13A1 correlated with a higher risk of ADHD. We are using a mouse model to understand the normal role of the gene and to determine the effects on motor function and behavior. Knockout Atp13a1(-/-) mice die before birth; therefore, we were only able to test heterozygous Atp13a1(+/-) and wild type Atp13a1(+/-) mice in our neurobehavioral test battery. Results from two tests are reported here. We found a significant gene x sex interaction for total activity in the open field locomotor activity test and during multiple intervals of the 60 min. test. Heterozygous female Atp13a1(+/-) mice had significantly higher activity than wild type females. However, heterozygous male Atp13a1(+/-) mice had significantly lower activity levels compared with wild type males (P < 0.05). We found a significant gene x sex interaction on two days of testing and a significant effect of sex on two days of testing. Heterozygous Atp13a1(+/-) females had longer latencies to fall on Days 1 and 3 compared with all other groups (P < 0.05). There was also a main effect of sex with females out-performing males on Days 1 and 5 of testing (P < 0.05). Overall, all mice showed evidence of motor learning over the five days of testing. This indicates normal motor function in both wild type and heterozygous mice.

**1363 A Lack of Changes in the Transcriptomic Response in the Hippocampus or Amygdala after Developmental Exposure to Mild Variable Stress**


Early developmental exposure to stress has been reported to influence learning and memory mechanisms. Changes in the gene expression of brain-derived neurotrophic factor (Bdnf), Ca2+/calmodulin-dependent protein kinase II alpha (Camk2a), and cyclic adenosine monophosphate (cAMP) response element binding (Creb1) have been associated with learning in rodent models. We utilized perinatal exposure to a variable stress paradigm to examine changes in learning and expression of these gene targets after trace fear conditioning. Pregnant Long Evans rats were exposed to an unpredictable series of mild stressful events, which had previously been shown to increase maternal corticosterone levels. These nonchemical factors were presented daily from gestational day (GD) 13 through GD 21 with a postnatal group that also included maternal separation. Starting at postnatal day 97, male and female adult offspring were trained with a trace fear conditioning (TFC) protocol whereby rats were exposed to a compound cue (light and tone) followed by 30 seconds (trace period) and a mild foot shock (1mA, 0.5 seconds). Five paired training sessions occurred on the training day. Hippocampus and amygdala were removed and flash frozen at 3 or 6 hours after TFC. All treatment groups displayed learning during context testing. Quantitative RT-PCR data generated from hippocampus or amygdala showed a lack of significant change in the expression of Bdnf, Camk2a, or Creb1 at either time point, or for either sex, regardless of stress exposure. This lack of change may indicate that these genes are influenced at different time points than when these tissues were collected, or that other gene targets could be responding to this learning task for these rats. On-going research will examine a wider range of RNA targets using RNAseq in adult rats that have completed TFC compared to controls that have not undergone testing, to isolate RNA targets that are influenced by TFC. Additional work will examine treatment-related changes in the offspring of dams concurrently exposed to manganese and variable prenatal stress, including transcriptional changes in the hippocampus and amygdala of adult rats after learning the TFC task. This abstract does not necessarily reflect US EPA policy.
1364 Early-Life Lead Exposure Increases µ-Opioid Receptor Levels in the Juvenile Rat Brain: Implications for Opioid Addiction


Opioids and cocaine are two of the main drugs of abuse that contribute to death by overdose. In the United States, from 2015–2016, overdose deaths from these drugs increased by 21%. Opioid-related overdoses accounted for 66% of the cases and cocaine overdose increased by 52% (Seth et al, 2018). The juvenile period is a time in which humans are particularly driven to seek rewards and to engage in drug-seeking and risk-taking behaviors. In the brain, activation of µ-opioid receptors is directly involved in these behaviors and µ-opioid receptor play an important role in opioid use disorders. Previously, we have shown that chronic early life exposure to lead (CELE) sensitizes animals to cocaine an effect mediated by an increase in D1-dopamine receptors. In this study, we examine the effect of CELE on µ-opioid receptor levels in the brain of juvenile rats. Our CELLE protocol has been extensively described over the last 25 years. This exposure paradigm results in blood lead levels in juvenile animals (28 days of age) as follows: control males: 1.71 ± 0.07 ug/dL (n=54); lead-exposed males: 19.9 ± 0.49 ug/dL (n=61); control females: 1.78 ug/dL (n=40); lead-exposed females: 24.4 ± 1.1 (n=39). To examine µ-opioid receptor levels in the brains of juvenile animals, we used quantitative autoradiography with the specific µ-opioid receptor agonist ligand [3H]-D-Ala2-MePhe4-Gly-ol2 enkephalin. Analysis of [3H]-D-Ala2-MePhe4-Gly-ol2 enkephalin specific binding to µ-opioid receptor was performed in the basolateral amygdala, hypothalamus, midthalamus, stria medullaris of the thalamus, lateral posterior thalamus, nucleus accumbens, prefrontal nucleus, and dorso-lateral geniculate nucleus of male and female rats. Statistical analysis indicates that lead-exposed male and female rats had significant increased levels of µ-opioid receptor than controls except in the hypothalamus of males and stria medullaris of the thalamus in females. Our results indicate that CELLE increases brain µ-opioid receptor levels in lead-exposed rats and this may be a risk factor for drug-seeking and risk-taking behaviors in juveniles. NIEHS grant Number E006189-24 to TRG.

1365 Altered Sterol Homeostasis during Neurodevelopment, In Vivo and In Vitro, Is a Common Target for Benzalkonium Chloride Disinfectants


Lipids are critical for neurodevelopment; thus, disruption of lipid homeostasis by environmental chemicals is expected to have detrimental effects on this process. We have demonstrated that the benzalkonium chlorides (BACs), a class of commonly used disinfectants, alter cholesterol biosynthesis and lipid homeostasis in neuronal cells, in a manner dependent on the alkyl chain length. However, the ability of BACs to reach the neonate brain and alter sterol and lipid homeostasis during neurodevelopment has not been characterized. Moreover, the impact of BACs on neurodevelopment has not been fully elucidated, although increased incidences of neural tube defects following in utero exposures has been observed. Therefore, we hypothesize that BACs would affect neurodevelopmental processes by altering sterol and lipid homeostasis. Damas were fed BACs of different alkyl chain lengths (C12 and C16) at 120 mg/kg/day via diet for one-week prior to mating and throughout gestation. BAC exposure led to an increase in sterol and lipid homeostasis was assessed using targeted and untargeted mass spectrometry methods and RNA sequencing. BACs were found in the neonate brain at low nM concentrations, which resulted in decreased total sterol levels and altered lipidome. BAC C12 treatment resulted in the differential expression of 507 genes, whereas BAC C16 altered the expression of 139 genes. Cholesterol biosynthetic genes were significantly upregulated, which is consistent with the inhibition of cholesterol biosynthesis by BACs. Changes to genes in lipid metabolism were also consistent with identified lipidomic changes i.e. significant decreases in levels of triglycerides and diglycerides. To examine effects of BACs on neurodevelopment, neural precursor cells were isolated from the mouse brain at embryonic day 14.5, cultured to neurospheres, and treated with either BAC at 10-100 nM. Neurosphere diameter was reduced to approximately 50% of the control, which was attributed to a loss of proliferative cells (Ki67+/DAPI+) rather than a decrease in proliferation (EdU+/Ki67+). Inhibition of cholesterol synthesis was also observed in treated neurospheres. Overall, the effects of BACs on sterol and lipid homeostasis and neurosphere proliferation support our hypothesis and prompt further investigation into the developmental neurotoxicity of BACs.

1366 The Placenta as a Potential Target of Neuroendocrine Disruption: A Comparison of Brominated and Organophosphate Flame Retardants

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Chemical flame retardants (FRs) are commonly applied to consumer products including foam-based furniture and infant products, textiles, and electronics. Widespread human exposure and accumulating evidence of endocrine dis-
rption have raised concerns regarding the possible toxicity of these chemicals, particularly on the developing brain. Throughout gestation, the placenta is a critical coordinator of fetal growth and development, including neuro-development. We have shown in rats and humans that FRs accumulate in placental tissue; in some cases, to a greater degree in male-associated placentas than female-associated placentas. Given the important role the placenta plays in fetal programming, disruption of placental function may be a critical but underappreciated mechanism by which FRs induce changes, including sex-specific changes, in brain and behavior. Here we compare the gene expression profiles of male and female-associated placentas collected from Wistar rat dams gestationally exposed to one of three different FR mixtures. One consisted of a mixture of polybrominated diphenyl ethers (PBDEs), an important class of FRs that, although largely phased out, are strongly associated with behavioral and cognitive impairments in humans. The other two FR mixtures contained more recently introduced brominated and organophosphate ester (OPE) compounds. Using a combination of transcriptomic approaches, we identified several putative pathways and/or putative FR targets that were altered by FRs, including endocrine, inflammatory and neurotransmitter signaling pathways. Some of these mRNA expression changes were sex-specific, with upregulation of genes like Esr1 and Ar present in female-associated placentas and Tdo2 and Htr2a in male-associated placentas. Future work will continue to probe the hypothesis that sex-specific alterations in placental function could be a mechanism by which exposure to FRs alters neural developmental behavior and outcome.

1370 Perfluorooctane Sulfonate (PFOS) Exacerbates Microglial Responses to Brain Injury in Exposed Zebrafish Embryos
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Per- and Polyfluoroalkyl Substances (PFAS) are a class of manufactured organic compounds commonly found in repellent or stain-resistant products, food packaging, and clothing, and are also additives in some cleaning agents, detergents, and cosmetics. Due to their high structural stability, these ubiquitous global toxicants do not readily degrade and, consequently, are environmentally persistent and highly bioaccumulative. Perfluorooctane sulfonate (PFOS), a prevalent PFAS congener, is immuno- and neurotoxic in both developmental and adult contexts. However, it is not known whether PFOS exposure affects the development and function of microglia, the resident immune population in the brain. Microglia play an active role in neuronal pruning and clearance during development, are required for synaptic remodeling, and are primary responders to CNS insults such as pathogen invasion or injury. In the present study, we examined microglia migratory behavior and function in response to minor injury via puncture to the right hemisphere of the telencephalon in 3-day-old zebrafish raised in either 0.1% DMSO or 32μM PFOS. As a developmentally rapid and transparent model, transgenic zebrafish allow for real-time in vivo analysis of fluorescently-labeled cellular activity. Using transgenic fish with fluorescently-labeled macrophages, which includes microglia, we demonstrate that embryos raised in water containing 32μM PFOS have similar numbers of brain macrophages, though exhibit a significantly heightened inflammatory response to injury, as evidenced by an expanded inflammatory field surrounding as well as an increased macrophage recruitment. Additionally, macrophages in PFOS-exposed embryos had prolonged residency at the site of injury. This suggests both a sustained release of inflammatory mediators, such as cytokines, by neurons and cells in the surrounding microenvironment, an intensified adverse reaction to minor injury, or both. Our findings present an opportunity for brain macrophages to recover after clearance of damaged tissue. Together, these data reveal an amplified and persistent response by microglia to brain injury with PFOS exposure and support current evidence that PFOS is neurotoxic, as prolonged neuroinflammation is known to contribute to neurotoxicity and drive neurodegeneration.

1371 Neuroactive Compound Alter Neural Network Formation Measured in Micro electrode Arrays with Potencies Lower Than Median Toxcast Potencies
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Thousands of chemicals with human exposure potential have not been evaluated for developmental neurotoxicity (DNT) due to resource limitations. This has driven efforts to develop efficient screening approaches to identify and prioritize compounds with the potential to cause DNT. One promising approach is assessment of chemical effects on neural network formation (NFA) using cultures grown on microelectrode arrays (MEAs). In a current study, 137 unique compounds were evaluated for their ability to disrupt formation of cortical networks grown on MEAs. Chemical exposure was initiated 2 hr following cell plating and continued for 12 days in vitro (DIV). Network activity was recorded on DIV 5, 7, 9 and 12, with cell viability assessed at DIV 12. Of the 137 unique compounds tested, 53 did not alter the connectivity of any network parameter. The remaining 84 compounds affected at least one parameter of network development, with 46 showing selective effects defined by ≥3x more potent EC50 values for effects on network formation vs viability. Assay results were highly reproducible; results with nine compounds tested twice as biological replicates were qualitatively 100% concordant. Tipping points, i.e., biological threshold concentrations that reflect effects on multiple NFA parameters, were determined for 61 compounds, and tended to be similar to or lower than the minimum EC50 (typically within less than 1 log10EC50). To characterize the value added by the NFA MEA to available high-throughput screening assay data, EC50 values for effects on network parameter were compared to the range of EC50 values observed in ToxCast assays. Generally, the NFA EC50 values for neurotoxic (and potentially developmentally neurotoxic) compounds were less than the interquartile range for ToxCast assay EC50 values for the same compounds, whereas compounds lacking evidence for neurotoxicity had EC50 similar to the median EC50 values in other ToxCast assays. These results indicate that NFA screening may identify the nervous system as a sensitive endpoint compared to other ToxCast assays, and demonstrate that data from the NFA could be used to screen and prioritize compounds for additional testing. Disclaimer: This abstract does not reflect policy of the US EPA.

1372 Maternal Exposure to Organophosphate Flame-Retardants and Anxiety-Like Behavior
Endocrine disrupting compounds (EDCs) are compounds found in our environment that interrupt typical endocrine function. A particular group of EDCs are flame-retardants due to their interaction with steroid and nuclear receptors in vitro investigations. Humans are consistently exposed to flame-retardants daily as they are used in everyday items such as plastics, clothing, toys, and electronics. In the past, polybrominated diphenyl ethers have been used; however, since 2004, they have been replaced with organophosphate flame-retardants (OPFR) as the major flame-retardant chemical. The effects of maternal or developmental exposure to OPFR on behavior are currently underexplored. Yet, one such maternal exposure study in rodent models utilizing a commercial flame-retardant mixture containing OPFR reported significant differences in open arm entries on the elevated plus maze (EPM) in females (Pataisal et al., 2013, J Biochem Mol Toxicol). Here we evaluate anxiety-like behavior in the open field test (OFT), as well as, the EPM in male and
female offspring that were maternally exposed to OPFR or oil controls. Males that were maternally-exposed to OPFRs had significantly reduced time of exploration in the center zone of the OFT, a marker of anxiogenic-like behavior, relative to their same-sex controls t(28) = 2.128, p = .042. No significant differences were found in center zone exploration time in maternally-exposed OPFR females relative to their oil controls (t(25) = -1.018, p = .319. Interestingly, experimental females displayed anxiety-like behavior as evidenced by a decreased number of entries in the corners of the OFT, t(25) = 3.556, p = .002. In contrast, we saw no effect in experimental males on number of corner entries t(29) = - .443, p = .661. We also assessed behavior in the EPM. Similar to outcomes in the OFT, maternally-exposed OPFR males had significantly reduced percent time in the open arms compared to same-sex controls (t(28) = 2.096, p = .045. However, we did not see an effect in females on this measure t(25) = .579, p = .567. Our research illustrates that there are sex-dependent effects of OPFR exposure on exploratory behaviors in a mouse model as was previously found in a rat model. Future studies will evaluate other behavioral measures such as the Light/Dark box, social interactions, the effects of high-fat diets, and the receptor-mediated mechanisms underlying the sex differences.

### 1373 Integration of Genomic and Metabolomic Data Streams in an In Vitro Neuronal Development Model

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A microelectrode array-based assay (MEA) has been developed in rat primary cortical cultures for screening and prioritization of chemical effects on neural network formation. In this study, pathway-based transcriptomic and metabolomic methods were utilized to complement this assay and identify molecular events involved in adverse outcomes associated to neural network disruption. Gene expression and metabolomic alterations were determined after exposure to two DNA synthesis inhibitors (Cytosine Arabinoside and 5-Fluorouracil), two voltage-gated sodium channel (VGSC) modulators (Deltamethrin and Cypermethrin), a dopamine receptor antagonist (Haloperidol) and a glutamate receptor antagonist (Domoic Acid). Doses (0.1-3μM) were selected based on critical concentrations at which the neural networks switched from recovery to non-recovery trajectories based on the MEA results. Global transcriptomic response for the DNA synthesis inhibitors repressed gene response, consistent with robust impacts on DNA synthesis, while the dopamine receptor antagonist showed a dose-dependent increase in gene expression. By contrast, exposure to VGSC modulators and the glutamate receptor agonist tended to increase gene expression. Regardless of chemical class, "Axonal Guidance Signaling" was a significantly altered transcriptomic pathway identified for all chemicals excluding Haloperidol, highlighting a shared disruption in neural development. Metabolomic analysis indicated twelve altered metabolites common to all tested chemicals related to neuronal, developmental, and psychological disease. Further, "Activation of mRNA charging" was the most significantly perturbed metabolomic pathway with all chemicals, indicating an impact on a general translational response in rat cortical neurones. Overall, our combined findings across MEA, transcriptomic, and metabolomic responses consistently reinforce each other, suggesting that the molecular endpoints may serve as a complement to the functional MEA assay. Importantly, these results provide data to build an adverse outcome pathway network based on neural development disruption. This abstract does not necessarily reflect US EPA policy.

### 1374 Adolescents’ Methymercury: Effects on Sustained Attention and Retention and Interactions with d-Amphetamine

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Some recent studies of human exposure have shown that exposure to methylmercury (MeHg) may result in attention deficits, but an experimental model of this has not been produced. Prolonged effects of adolescent exposure to MeHg on sustained attention and short-term retention were examined by exposing adolescent male Long-Evans rats to 0, 0.5, or 5 ppm MeHg throughout adolescence and testing them in adulthood, on a two-choice visual signal detection task. Rats were trained to respond on one lever in the presence of a 0.3” signal (hit) or an alternative lever in its absence (correct rejection). Stimuli were presented randomly within a 0.3-74 second window. To examine retention, the response lever was made available after a random delay of 0.3-29.3 seconds. Acute effects of d-amphetamine were also examined in order to determine the sensitivity of MeHg exposed animals to dopamine modulation. Animals exposed to 0.5 ppm MeHg had lower overall accuracy, and this was attributed to a selective decrease in the hit rate. Amphetamine reduced overall accuracy across post-stimulus delays by decreasing the hit rate, especially at long delays. This occurred in all exposure groups. Further, amphetamine decreased correct rejections (increasing false-alarms) for animals exposed to the low dose of MeHg. These findings suggest that MeHg alters attention and retention and these effects are sensitive to modulation by dopamine agonists. Interestingly, previous reports of a nonlinear dose-effect curve that has previously been reported, in which the 0.5 ppm exposure group is more sensitive than the 5 ppm group, was replicated here.

### 1375 Autism Spectrum Disorders (ASD) and Cerebral Palsy (CP) as Neurodevelopmental Disorders in Children in Ibadan, Nigeria: Pb and Se in Focus

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The level of exposure to toxic metals is on the increase worldwide. Recent studies have shown that some of the toxic metals are associated with neurological diseases and affect essential elements and micronutrients absorption. Autism spectrum disorders (ASDs) and cerebral palsy (CP) are neurodevelopmental disorders that affect millions of children worldwide and diminish quality of life. Toxic element like Pb have been shown to affect essential element and micronutrient absorption and their interaction have been shown to have toxic effect. This study was conducted to investigate the levels of toxic metal (Pb) and micronutrients (Se) and their roles in aetiologypathogenesis of ASD and CP in Ibadan, Nigeria. 8 children with ASD and 8 CP and 16 age-sex matched neurotypical children as controls were recruited for this study. All diagnoses were made by the pediatric neurologist. Plasma levels of Pb, and Se were analyzed using ICP-MS method. Ethical clearance was obtained from UCH/UI ethical review committee. Results were analyzed using Students t-test. The analysis of the questionnaire revealed that the gender difference was not significant in the children (p=0.579). The children's mean Se level was significantly lower in ASD than in CP (14.45±4.29) compared to controls (32.51±9.71) and control subjects (58.26±11.69). Moreover, chemical concentration in neurotypical children showed Pb level in ASD (7.92±1.30) and CP (10.38±1.45) compared to control (6.83±0.72) while Se level was significantly reduced in subjects (0.37±0.05; 0.30±0.02) compared to control (0.57±0.02). The central nervous system is prone to the deleterious effect of Pb: thus its effect is more pronounced in children where it interferes with the normal brain development. On the other hand, Se has a neuroprotective effect due to its antioxidant and anti-inflammatory properties. The important redox properties of Se may have been compromised by the reduced Se level observed in ASD and CP children in this study may contribute to the pathophysiology of these neurodevelopmental disorders. It may be concluded that the burden of lead and reduced Se status in these participants contributed to the consequences of the disorders.

### 1376 Assessment of Neurotoxic Potential of 90 Blinded Compounds Using Zebrafish Embryos

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Developmental toxicity and neurotoxicity of 90 blinded compounds containing known neurotoxicants (DNT/NT) and compounds with undetermined neurotoxic potential (e.g. flame retardants, bisphenol A analogs) were assessed using zebrafish embryos. To evaluate developmental toxicity, chemicals were tested at 5 to 8 concentrations and a teratogenic index (TI) was then calculated (log dose ratio = TICM (median effect concentration) and TELC (lethal effect concentration) on morphological alterations). For neurotoxicity evaluation, embryos were treated at 3 dpf (days post fertilization) with 5 doses, with the lowest concentration where morphological effects appeared selected as the highest concentration, and after 48 hours of exposure, locomotor activity was analyzed as indicative of neurotoxicity. Larvae from the developmental toxicity assay were analyzed for internal compound concentrations to determine the real concentration at which toxic effects were induced. Moreover, chemical concentration in the medium was also determined. After compound unblinding, within the 38 DNT/NT compounds, 26 (68.4%) were detected as active in zebrafish embryos, 54% of the active chemicals being neurotoxic and the other 46% toxic. The remaining 12 compounds did not induce any toxicity. However, the bioavailability data showed that in 11 out of the 12 cases the internal concentration did not reach 10 μM because of the limited uptake, instability or precipitation. In addition, 33 out of 45 (76.7%) of compounds with unknown DNT/NT were the most neurotoxic and 31 of these were toxic as well. This study demonstrates the utility of zebrafish embryos as a prioritization method to identify novel compounds with neurotoxic potential.
**1377 Social Behavioral Effects in Prairie Voles Perinatally Exposed to Firemaster 550**

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The prairie vole (Microtus ochoaegaster) is a uniquely valuable model organism used to study complex social behavior. This species readily form social bonds and spontaneously display social monogamy, bi-parental care, and partner preference; behaviors not seen more traditional laboratory rodent models such as rats or mice. Our studies demonstrate the utility of the prairie vole for investigating the impact of chemical exposures on social behavior. Healthy social interactions and the ability to form stable social attachments are important for mental health and impairments are common characteristics of some mental health disorders. The incidence of neurodevelopmental disorders in children is rapidly rising raising speculation that developmental exposure to environmental contaminants may be contributory. Firemaster 550 (FM550) is one of the most prevalent flame retardant (FR) mixtures used in foam-based furniture and baby products. We and others have published evidence of developmental neurotoxicity and sex-specific effects of FM550 on anxiety-like and exploratory behaviors in rats and zebrafish suggesting impacts on social behavior. To test this hypothesis, we investigated the impact of perinatal FM550 exposure on a range of social behaviors in prairie voles. Dams were exposed to 0, 500, 1000, or 2000 μg of FM500 via subcutaneous injections throughout gestation, and pups were directly exposed to the same dose beginning the day after birth until weaning at three weeks of age. Tasks performed on the offspring of both sexes were open field, novel social, social preference, social recognition, novel object recognition, and partner preference. Effects were dose responsive and sex specific, with females more affected than males. Behavioral effects included elevated anxiety, decreased social interaction, decreased exploratory motivation, and altered social preference for novel versus partner animals. Future studies will probe the possible mechanisms by which these effects arise. These data support the hypothesis that developmental FR exposure impacts the social brain and demonstrate the value of the prairie vole for assessing chemical effects on sociality and attachment.

**1378 Sex-Specific Effects of Polychlorinated Biphenyls (PCBs) on Hippocampal Dendritic Arborization in Weanling Mice**


Early-life exposures to environmental chemical contaminants are associated with increased risk of neurodevelopmental disorders (NDDs). PCBs are environmental contaminants that are resistant to degradation and globally distributed with high potential for human exposure. We previously identified elevated levels of specific PCB congeners in the serum of pregnant women enrolled in the MARBLES study, all of whom are at increased risk of having a child with a NDD. We hypothesized that developmental exposure to a PCB mixture that proportionally mimics the 12 most prevalent PCB congeners in the MARBLES maternal serum would alter neuronal maturation in the hippocampus via modulation of dendritic arborization. Wild type mouse dams were exposed daily to vehicle (peanut oil) or the MARBLES PCB mix (0.1, 1.0, or 6.0 mg/kg) in peanut butter beginning 2 weeks prior to mating and continuing throughout gestation and lactation. Offspring weaned at postnatal day (PND) 21 were euthanized at PND 28 to collect brains for Golgi staining. The basal dendrites of Golgi-impregnated CA1 pyramidal neurons were visualized on a confocal microscope and traced using Neurolucida software for Sholl and morphometric analyses. There was an overall significant effect of PCB exposure on dendritic complexity, the total number of dendrites, and mean dendritic length, with a trending effect on the number of dendritic tips. Significant sex by dose interactions were observed for cell body area, total number of nodes, and total number of dendritic tips, indicating the effects of developmental PCB exposure may be sex-specific. Dendritic effects exhibited non-monotonic dose-response relationships: in female offspring increased mean dendritic length and tips per dendrite observed only in the 0.1 mg/kg dose group, whereas in male offspring, decreased total dendrite number, nodes, tips per dendrite, and cell body area were observed in the 1.0 mg/kg dose group. These data indicate that developmental PCB exposure causes sex-specific changes in neuronal maturation in the hippocampus. These findings underscore the need for further investigation to identify the biological reasons for the differential vulnerability of the female vs. male brain in PCB developmental neurotoxicity. Supported by NIHS (RO1 ES014901; K99 ES029537 KPK; T32 ES007059 SS; P01 ES011269 PLJ).

**1379 Assessing the Developmental Toxicity and Developmental Neurotoxicity of 26 Organophosphorus Pesticides Using a Zebrafish (Danio rerio) Larval Assay**


The US Environmental Protection Agency is evaluating methods to screen and prioritize organophosphorus (OP) pesticides for developmental toxicity and developmental neurotoxicity (DNT). One of those methods uses larval zebrafish. Developmental toxicity in zebrafish embryos/larvae is defined as lethality, non-hatching, or dysmorphology (e.g. curved body axis, edema, non-inflation of swim bladder). DNT is defined as behavioral alterations in the light/dark locomotor assay, in which the locomotor response to light stimuli under tandem light and dark conditions in a 96-well plate is quantified using a video tracking system on 6 day post fertilization zebrafish larvae. Twenty-six OP pesticides were tested for their developmental toxicity and DNT potential by exposing zebrafish embryos/larvae to the pesticide at several concentrations (≤ 100 μM nominal concentration) during the first 5 days of development (daily renewal of chemical), followed by 24 hours of depuration. Behavioral testing was conducted at six days after fertilization followed by a visual assessment of the larvae for lethality, hatching status, and dysmorphology. Behavior was only analyzed for normal animals. Five of the pesticides produced no effect in either assay: Chlorothoxyx, Dimethoate, Fosthiazate, Methamidophos, and Trichlorfon. Slightly more than half (14/26) of the OP pesticides were developmentally toxic, and half (13/26) were neurodevelopmentally toxic to the developing zebrafish. Six of the OP pesticides (Profenofos, Acephate, Coumaphos, Diclofop, Ethoprop, and Terbufos) showed significant specificity for DNT in that they were neurodevelopmentally toxic to the zebrafish larvae at doses ≥ 3 orders of magnitude below the doses that elicited death of larvae. This suggests that some of these OP pesticides could affect brain development in zebrafish at concentrations below toxic levels for embryos. This abstract may not necessarily reflect official Agency policy.

**1380 Behavioral Consequences of Retinoid Disruption during Embryonic Development in the Zebrafish**

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A variety of environmental contaminants are known to cause neurobehavioral toxicity after developmental exposure(s), although more work is needed to understand the relevant adverse outcome pathways. Our previous work with the zebrafish model has shown that excess vitamin A (retinol) or exposure to valproic acid (VPA) which disrupts retinoidic acid (RA), alters behavioral function in larval zebrafish and into adulthood. This suggests that RA signaling may be a key adverse outcome pathway. Recent screening of Tox21 compounds has revealed a number of chemicals which may transactivate the retinoic acid receptor, including select pesticides (see below). Further work is needed to link their putative retinoid activity to adverse behavioral outcomes. The present study measured the behavioral effects of embryonic exposure to these compounds in larval and adult fish. Zebrafish embryos were exposed to vehicle (DMSO), chlorothalonil (CTN, 10-100μM), imazalil (IML, 0.1-1μM), endosulfan-I (ESF, 0.1-1nM) or buprofezin (BPF, 0.3-5μM) from 5-120 hours post-fertilization (hpf). Doses fell below the threshold for overt toxicity. Larval motility was assessed at 144hpf. Adult testing took place at 5-7 months. Each of the selected compounds significantly altered larval motility. The high doses of BPF and IML reduced motility under both light and dark conditions, while the lowest dose of IML increased motility regardless of lighting condition. The highest dose of CTN reduced motility in the dark, as did the lowest dose. The highest dose of ESF enhanced motility in the dark. Preliminary data from adult fish suggest alterations in affective functions in adulthood. CTN, ESF and IML appear to disrupt the dive response in the novel tank test, while BPF appears to enhance this response. As more fish and behavioral assays are added to this toolkit, the possible outcome pathways are expanded. The long-term consequences of these exposures may be clarified. Supported by the Duke Superfund Research Center (ES010356).
Due to their ubiquitous use, there is major concern regarding the use of flame retardants, specifically organophosphate flame retardants (OPFR) and their actions as endocrine disrupting chemicals (EDC). OPFR concentrations are detectable in urine samples and breast milk from pregnant and nursing women. The effects that maternal exposure of OPFR have on offspring metabolism is unknown. This study will provide evidence to the impact of OPFR on hypothalamic and liver gene expression in the neonate of a wild-type mouse. Breeding pairs were established using virgin females and assigned to either oil or an OPFR mixture (triphenyl phosphate, tricresyl phosphate, and tris(1,3-dichloro-2-propyl)) at 1 mg/kg each from gestational day 7 to postnatal day 14. Total litter weight was taken at birth (day 0) and basal hypothalamic and liver tissue was collected from one female and one male along with individual body weights. On postnatal day 14, the same tissue was collected from a second female and male pup from each litter along with individual body weight. RNA was extracted and processed for measurement of hypothalamic and liver genes using quantitative real-time PCR. In the hypothalamus, we found age-dependent effects for Pomc, Npy, Agpp, Cart, Esr1, Esr2, Kiss1, Lepr, Insr, Ghsr, Pparg, Bdnf, Pdyn, and Tac2 and effect of OPFR exposure on Esr1, Pparq, and Tac2 with interactions of OPFR, age, or sex in Tac2, Pdyn, Bdnf, Pparq, Kiss1, Esr1, and Cart. Specifically, OPFR exposure increased Bdnf, Pdyn, Tac, Esr1, and Pparq and decreased Kiss1 expression in females on Day 14 with no effects in males. We are currently examining liver gene expression from the same neonates focusing on genes involved in glucose, fatty acid, and triglyceride homeostasis and nuclear receptors. Collectively, these data suggest that maternal OPFR exposure has age- and sex-dependent effects of neonatal gene expression. Supported by R25ES020721 and R21ES027119.

1338 Behavioral Impairments of Infant and Adult Mice Exposed to 2,3,7,8-Tetramethyl-benzofuran In Utero and via Lactation

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In utero and lactational exposure to environmental chemicals has been reported to induce behavioral abnormalities and cognitive function impairment in humans and laboratory animals. We have previously shown that maternal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin, the most toxic congener in the group of chlorinated dioxins and furans, congeners, induced activation of aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor, in various tissues, including liver and brain tissues, and led to atypical ultrasonic vocalization (USV) in infant mouse offspring. Polybrominated dibenzofurans (PBDFs) have been detected as impurities in brominated flame retardants; however, little is known about the effects of perinatal PBDF exposure on brain function. Therefore, we assessed whether PBDF exposure affects infant and adult behavior by analyzing mouse offspring born to dams treated with 2,3,7,8-tetrachlorodibenzo-furan (TCDD) by gavage at 0.9 or 45 μg/kg b.w. on gestational day 12.5 (hereafter referred to as control, L-TCDF, and H-TCDF groups, respectively). On postnatal day (PND) 1, the mRNA and protein expression levels of Cyp1a1, an AhR target gene, in the liver were markedly increased in the L-TCDF and H-TCDF groups, in a dose-dependent manner, indicating that AhR activation occurs at these exposure doses. Next, USVs emitted by infant offspring on PNDs 3-9 were recorded for 1 min in a sound-attenuated chamber. Total USV duration was significantly reduced in the H-TCDF group compared to that in the control and L-TCDF groups, indicating lower adaptivity to novel environments. These results show behavioral impairment in infant and adult stages induced by perinatal TCDD exposure, and suggest USV and adaptivity as useful endpoints to assess the developmental neurotoxicity of chemicals.
Genetic Differences in Neurological Development in Mice Exposed to Benzo[a]pyrene Exposure during Late Gestation and Lactation

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Traffic-related air pollution (TRAP) is a mixture of chemicals with the predominant forms being polycyclic aromatic hydrocarbons (PAHs) (US EPA 2017). Although the effects of TRAP on lung function and heart health are well known, there is emerging evidence that TRAP and PAHs also harm the developing brain. Genetic variation can increase an individual’s resistance or vulnerability to environmental exposures. Based on previous work indicating that Cyp1a1(-/-) knockout mice were more susceptible to fetal loss and DNA adducts following maternal exposure to the PAH benzo[a]pyrene, we extended our findings to look at neurobehavioral outcomes. Pregnant mice with the Cyp1a1(+/-), Cyp1a1(+/s) or Cyp1a1(-/-) genotypes were treated with 10mg/kg BaP in corn oil-soaked food from gestational day 10 (GD10) through weaning at postnatal day 25 (P25). Offspring were tested for surface righting reflex at P5, P7 and P10 and in the negative geotaxis test at P7, P10 and P14 to assess neurological development in the neonatal period. We found no significant differences based on treatment or genotype in the surface righting reflex test at P5, P7 or P10, however, there was a significant main effect of treatment at P7. BaP-exposed mice were significantly faster than corn-oil-treated controls (P < 0.05). In the negative geotaxis test, there was a significant main effect of genotype at P7 with wild type Cyp1a1(+/-) mice having the longest latencies to turn compared with heterozygous and homozygous knockout mice. At P10, there was a significant gene × treatment interaction (P < 0.001). BaP-exposed heterozygous mice took significantly longer to turn compared with corn-oil-treated mice and BaP-exposed wild type and knockout mice. There were no significant differences at P14. In summary, these findings suggest there is a gene-dose effect for negative geotaxis but no delay in development of the surface righting reflex. Instead, the shorter latencies in BaP-exposed mice are suggestive of a hyperactive phenotype.

Developmental Exposure to Polychlorinated Biphenyls (PCBs) Induces Increased Nerve Density and Inflammation in the Bladder and Voiding Changes in Young Adult Mice

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Lower urinary tract symptoms (LUTS) affect people of all ages. Bladder inflammation is a common feature, yet underlying mechanisms driving the onset and progression of LUTS are poorly defined. Developmental exposure to environmental neurotoxins is implicated in the pathogenesis of many neurodevelopmental diseases, but whether they increase individual LUTS risk remains unknown. We tested the hypothesis that developmental exposure to polychlorinated biphenyls (PCBs) increases bladder inflammation, nerve density and contributes to abnormal voiding in young adult mice. Wild type mice were exposed through gestation and lactation to 0, 0.1, 1 or 6 mg/kg/d PCB mix via the maternal diet. The PCB mix proportionally mimics the 12 most prevalent PCB congeners in serum of women at increased risk of having a male child with a neurodevelopmental disorder. Bladder tissue for immunohistochemistry, bladder metrics and spontaneous void spot assays were collected from postnatal day 28-31 mice. Effects of PCBs on bladder were sex-and dose-dependent. Compared to vehicle controls, PCBs (0.1 mg/kg/d) increased CD45-positive immune cells in the bladder in both sexes. PCBs (1 mg/kg/d) also altered immune cell localization by increasing CD45-positive cells within bladder epithelium in male mice. Inflammation can lead to nerve hyperplasia, therefore we examined abundance of beta tubulin-positive nerve fibers adjacent to epithelium. This was increased by PCBs (6 mg/kg/d) in males, and this effect was greater in males vs. females. PCBs also altered bladder metrics and voiding. PCBs (6 mg/kg/d) increased bladder volume, and PCBs (1 and 6 mg/kg/d) decreased the number of urine spots sized 0-0.1cm in male mice. Urine creatinine and protein were unaltered, but urine ATP was increased from postnatal day 28-31 mice. Effects of PCBs on bladder were sex- and chemistry, bladder metrics and spontaneous void spot assays were collected at weaning at postnatal day 25 (P25). Offspring were tested for surface righting reflex at P5, P7 and P10 and in the negative geotaxis test at P7, P10 and P14 to assess neurological development in the neonatal period. We found no significant differences based on treatment or genotype in the surface righting reflex test at P5, P7 or P10, however, there was a significant main effect of treatment at P7. BaP-exposed mice were significantly faster than corn-oil-treated controls (P < 0.05). In the negative geotaxis test, there was a significant main effect of genotype at P7 with wild type Cyp1a1(+/-) mice having the longest latencies to turn compared with heterozygous and homozygous knockout mice. At P10, there was a significant gene × treatment interaction (P < 0.001). BaP-exposed heterozygous mice took significantly longer to turn compared with corn-oil-treated mice and BaP-exposed wild type and knockout mice. There were no significant differences at P14. In summary, these findings suggest there is a gene-dose effect for negative geotaxis but no delay in development of the surface righting reflex. Instead, the shorter latencies in BaP-exposed mice are suggestive of a hyperactive phenotype.

Methylmercury is a potent neurotoxicant, however the mechanisms involved in its neurotoxicity are not elucidated. We recently found that the expression of TNF-α (tumor necrosis factor-α), an inflammatory cytokine, was specifically induced in the brains of mice, which administered with methylmercury causes neuronal damage. Thus, elucidation of the mechanisms involved in the induction of TNF-α expression could be a clue to reveal the mechanisms related to methylmercury-induced neurotoxicity. This study aimed to identify the responsible cells that involved in the induction of TNF-α expression by methylmercury in mouse brain and to approach the mechanisms. Increase of TNF-α mRNA level was observed in mice cerebrum cortex and cerebellum 7 days after the subcutaneous injection of methylmercury (25 mg/kg). We performed in situ hybridization using the brains and found that TNF-α expressing cells were observed in the cerebrum cortex and cerebellum. Next, immunostaining was performed using antibodies against GFAP or Iba1, which are marker proteins for astrocytes or microglia respectively. As a result, TNF-α expressing cells are positive for Iba1 staining but negative for GFAP staining. This indicates microglia are mainly involved in the induction of TNF-α expression by methylmercury in mouse brain. We next investigated the effect of methylmercury on TNF-α expression using primary mouse microglia. Exposure of the cells to methylmercury increased TNF-α mRNA level in a dose- and time-dependent manner. This increase was abolished by pretreatment with transcription inhibitor. These results suggest that methylmercury induces TNF-α expression in microglia via the activation of some transcription factors. It has been reported that transcription factor NF-kB and AP-1 are mainly involved in the induction of TNF-α expression in microglia. Exposure of the cells to methylmercury resulted in the phosphorylation of p65 and c-Jun, which are subunits of NF-kB and AP-1. However, transfection of the siRNA for c-Jun did not affect induction of TNF-α expression by methylmercury, while the siRNA for p65 partially suppressed the induction. Our result indicates that NF-kB is a transcription factor that partly involved in the induction of TNF-α expression by methylmercury in microglia.

Manganese is an essential metal that becomes neurotoxic at elevated levels. Yet, mechanisms by which manganese homeostasis is regulated in the brain are unclear. Loss-of-function mutations in SLC30A10, which codes for a cell-surface localized manganese efflux transporter expressed in the brain and liver, induce familial manganese neurotoxicity in humans, suggesting the major route of manganese excretion, manganese levels in the brain and blood under basal conditions. Further, while transport into bile is thought to be a major route of manganese excretion by methylmercury in mouse brain; we next investigated the effect of methylmercury on TNF-α expression using primary mouse microglia. Exposure of the cells to methylmercury increased TNF-α mRNA level in a dose- and time-dependent manner. This increase was abolished by pretreatment with transcription inhibitor. These results suggest that methylmercury induces TNF-α expression in microglia via the activation of some transcription factors. It has been reported that transcription factor NF-kB and AP-1 are mainly involved in the induction of TNF-α expression in microglia. Exposure of the cells to methylmercury resulted in the phosphorylation of p65 and c-Jun, which are subunits of NF-kB and AP-1. However, transfection of the siRNA for c-Jun did not affect induction of TNF-α expression by methylmercury, while the siRNA for p65 partially suppressed the induction. Our result indicates that NF-kB is a transcription factor that partly involved in the induction of TNF-α expression by methylmercury in microglia.

Activity of SLC30A10 in the Digestive and Nervous Systems Regulates Brain Manganese under Basal and Neurotoxic Conditions Respectively

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Manganese is an essential metal that becomes neurotoxic at elevated levels. Yet, mechanisms by which manganese homeostasis is regulated in the brain are unclear. Loss-of-function mutations in SLC30A10, which codes for a cell-surface localized manganese efflux transporter expressed in the brain and liver, induce familial manganese neurotoxicity in humans, suggesting the major route of manganese excretion by methylmercury in mouse brain; we next investigated the effect of methylmercury on TNF-α expression using primary mouse microglia. Exposure of the cells to methylmercury increased TNF-α mRNA level in a dose- and time-dependent manner. This increase was abolished by pretreatment with transcription inhibitor. These results suggest that methylmercury induces TNF-α expression in microglia via the activation of some transcription factors. It has been reported that transcription factor NF-kB and AP-1 are mainly involved in the induction of TNF-α expression in microglia. Exposure of the cells to methylmercury resulted in the phosphorylation of p65 and c-Jun, which are subunits of NF-kB and AP-1. However, transfection of the siRNA for c-Jun did not affect induction of TNF-α expression by methylmercury, while the siRNA for p65 partially suppressed the induction. Our result indicates that NF-kB is a transcription factor that partly involved in the induction of TNF-α expression by methylmercury in microglia.
1390 SLC30A10 Mutation Involved in Parkinsonism Results in Manganese Accumulation within Nano-Vesicles of the Golgi Apparatus Revealed by Synchrotron X-Ray Fluorescence Imaging


SLC30A10 is a cell surface protein involved in the efflux of Mn and protects the cell against Mn toxicity. Disease causing mutations block the efflux activity of SLC30A10, resulting in Mn accumulation. Determining the intracellular localization of Mn when disease-causing SLC30A10 mutants are expressed is essential to elucidate the mechanisms of Mn neurotoxicity. Here, using cryogenic nano-imaging (<50 nm resolution) indicated that Mn was trapped in single vesicles within the Golgi apparatus. Our results confirm the role of SLC30A10 in Mn efflux and the accumulation of Mn in cells expressing the disease causing SLC30A10-A105-107 mutation. Moreover, we identified sub-organellar Golgi nano-vesicles as the main compartment of Mn accumulation in SLC30A10 mutants suggesting interactions with the vesicular trafficking machinery as a cause of the disease.

Manganese (Mn) is an essential metal that can be neurotoxic when elevated exposure occurs leading to parkinsonian-like syndromes. Mutations in the SLC30A10 gene have been identified in new forms of familial parkinsonism. SLC30A10 is a cell surface protein involved in the efflux of Mn and protects the cell against Mn toxicity. Disease causing mutations block the efflux activity of SLC30A10, resulting in Mn accumulation. Determining the intracellular localization of Mn when disease-causing SLC30A10 mutants are expressed is essential to elucidate the mechanisms of Mn neurotoxicity. Here, using cryogenic nano-imaging (<50 nm resolution) indicated that Mn was trapped in single vesicles within the Golgi apparatus. Our results confirm the role of SLC30A10 in Mn efflux and the accumulation of Mn in cells expressing the disease causing SLC30A10-A105-107 mutation. Moreover, we identified sub-organellar Golgi nano-vesicles as the main compartment of Mn accumulation in SLC30A10 mutants suggesting interactions with the vesicular trafficking machinery as a cause of the disease.

1391 LRRK2 Kinase Activity Is Involved in Manganese-Induced Oxidative Stress, Inflammation, and Apoptosis in Microglia


Chronic exposure to excess manganese (Mn) induces a Parkinson’s disease (PD)-like neurological disorder referred to as manganism, however, the molecular mechanisms underlying Mn-induced neurotoxicity are not fully understood. The leucine-rich repeat kinase 2 (LRRK2) and its genetic mutations have been associated with the pathogenesis of familial and sporadic PD. LRRK2 is a multi-domain protein including GTPase and kinase domains. Several environmental toxicants such as rotenone have been reported to enhance LRRK2 kinase activity, indicating that environmental toxicants interact with LRRK2, contributing to LRRK2-induced PD pathogenesis. In the present study, we investigated if Mn increases LRRK2 kinase activity, contributing to Mn-induced toxicity in HMC3 microglia and RAW264.7 cells. We also tested if SLC30A10 mutations in single vesicles within the Golgi apparatus. Our results confirm the role of SLC30A10 in Mn efflux and the accumulation of Mn in cells expressing the disease causing SLC30A10-A105-107 mutation. Moreover, we identified sub-organellar Golgi nano-vesicles as the main compartment of Mn accumulation in SLC30A10 mutants suggesting interactions with the vesicular trafficking machinery as a cause of the disease.

1392 Urinary Cadmium and Cognitive Function

F. Scinicariello.

Gradual cognitive decline, usually affecting memory, is the hallmark feature of dementia and Alzheimer’s disease. Cadmium (Cd) is a widespread industrial and environmental pollutant, with tobacco and food being the primary sources. Cd has a long biological half-life (15-30 years in humans) mainly due to its low rate of excretion from the body and urinary excretion of Cd is assumed to mirror chronic exposure to Cd. To examine the association between urinary cadmium and cognitive functions. Multivariate linear regression were used to analyze the association of urinary cadmium with cognitive function in adults 60 and older, participants in the National Health and Nutrition Examination Survey cycles 2011–2012 and 2013–2014. Multiple cognitive domains were assessed: 1) verbal episodic memory assessed by the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) Word List Learning Test, the CERAD Word List recall Test, and the CERAD Saving Score; 2) semantic fluency, executive functions assessed by the Animal Fluency test; and 3) attention and executive function assessed by the digit symbol substitution test (DSS). The analyses were performed with urinary natural log-transformed Cd expressed as µg/g of urinary creatinine (InUCd) stratified by age group (60 - 69 years, and 70 years and older) with age years as continuous co-variate. Result: Decreased cognitive function domains were independently associated with increased InUCd in adults 70 and older, whereas the decrease in the age group 60 - 69 years was not statistically significant. For each InUCd there was a decrease in the verbal episodic memory tests CERAD-word list, recall (β = -0.70; 95% CI: -1.02, -0.38); CERAD-Delayed recall (β = -0.60; 95% CI: -1.21, -0.07); and CERAD-saving score (β = -0.85; 95% CI: -1.17, -0.35). For each InUCd there was a decrease in the animal fluency test (β = -1.20; 95% CI: -2.13, -0.26) and in the DSS (β = -1.20; 95% CI: -2.13, -0.26). This is the first study reporting an association of urinary cadmium, which is an indicator of cumulative body burden levels with poorer cognitive functioning in elderly aged 70 years and over using a nationally representative sample. Disclaimer: 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.

1393 Sex-Dependent Effect of Arsenic on Hippocampal Neuronal Apoptosis and Cognitive Impairment in Rats: Involvement of BMP and BDNF Signaling


Recent studies report a sex-dependent effect of arsenic on cognitive performances; however, the sex-dependent effect of arsenic on hippocampal neurons and learning-memory functions remain elusive. Therefore, in the present study, we were interested in identifying the effect of arsenic on hippocampal neurons of male and female rats. Here, we identified that arsenic exposure induced greater hippocampal neuronal apoptosis, marked by caspase-3 activation and Terminal deoxynucleotidyl transferase dUTP nick-end labeling, within the male rat brain compared to female. Correlating these observations with cognitive performances indicated a more severe reduction in transfer latency time and learning-memory ability for passive avoidance and Y-Maze tests respectively in arsenic-exposed male compared to female rats. Supportively, we noticed that noggin or recombinant BDNF supplementation reduced the arsenic-induced hippocampal neuronal apoptosis and cognitive loss, and here we further identified that arsenic caused a greater increase in BMP2/Smad and decreased BDNF/TkRk signaling as responsible for arsenic-induced hippocampal neuronal apoptosis and cognitive loss, and here we further identified that arsenic caused a greater increase in BMP2, BMP2P and p-Smad levels and decrease in BDNF and p-TkRk levels in arsenic-exposed male compared to female rats. 47 tadpoles were exposed stage III to IV to arsenic and then sacrificed at the age of 37 days. We noticed that noggin or recombinant BDNF supplementation reduced the arsenic-mediated loss in transfer latency time and learning-memory ability for passive avoidance and Y-Maze tests respectively in both the sexes. Overall, our study suggests that men may be more prone to arsenic-induced loss in cognitive performance and via increased BMP2/Smad and decreased BDNF/TkRk-dependent hippocampal neuronal apoptosis.

1394 Lead (Pb) Exposure Induces Biphasic Changes in Brain Development and Disrupts Thyroid Hormone Physiology in the Developing Tadpole Brain


Lead (Pb) poisoning during early development is associated with behavioral deficits, including low IQ. The specific neural mechanisms by which Pb causes these effects is still not fully understood. To address this issue, experiments assessed the effects of Pb on cellular and molecular mechanisms of brain development in Xenopus laevis tadpoles. We exposed stage III to IV tadpoles to Pb baths ranging from 10 ppb to 10,000 ppb and sacrificed them at various time points post-onset of treatment. Within the first few days of treatment,

1. Cadmium, a neurotoxic environmental compound, produces cognitive disorders, although the mechanism remains unknown. Cadmium induces a more pronounced cell death on cholinergic neurons from basal forebrain (BF), mediated, in part, by increase in Aβ and phosphorylated Tau protein levels, which may explain cadmium effects on learning and memory processes. Cadmium downregulates the expression of heat shock proteins (HSPs) HSP 90, HSP70 and HSP27, and of HSF1, the master regulator of the HSP pathway. This downregulation of HSP expression is observed in BF neurons from our laboratory using non-human primates in which we found dysfunctional but an intact nigrostriatal dopaminergic system suggesting that the neuropathology of chronic Mn exposure is different than that observed in PD. Ongoing studies aim to further characterize the behavioral effects and neuropathology of elevated Mn in SLC39A14-KO male and female mice.

1398 Identification of Accessible Cysteine Residues in Neuronal-Derived Sepiapterin Reductase as Targets of Methyl Mercury

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Methylmercury (MeHg) is an important environmental contaminant known to cause neurotoxicity. Our laboratories have examined the mechanism of action of MeHg using SK-N-MC cells, a cell line derived from a human brain neuroepithelium. In SK-N-MC cells, MeHg binds to sulfhydryl containing proteins, treatment of the cells with buthionine sulfoximine (BSO) a gamma-glutamylcysteine synthetase inhibitor which reduces levels of cellular glutathione (GSH), enhanced MeHg protein binding, BSO also enhanced MeHg cytotoxicity in the cells (LC50 = 3.1 µM and 3.8 µM in the presence of BSO, respectively). We identified sepiapterin reductase (SPR) as target for MeHg. SPR is an NADPH-dependent enzyme that catalyzes the reduction of sepiapterin to dihydrobiopterin (BH2), a precursor for tetrahydrobiopterin (BH4), a cofactor critical for enzymes mediating the biosynthesis of neurotransmitters. SPR also mediates redox cycling, catalyzing one electron reduction of reductase active chemicals including quinones; rapid reaction of these radicals with molecular oxygen generates reactive oxygen species. Using a recombinant human enzyme, MeHg was found to inhibit both the reduction of sepiapterin and quinone redox cycling (IC50 = 4.2 and 5.1 µM, respectively). Cysteine readily suppressed inhibition of SPR enzyme activities indicated that MeHg reacts with accessible thiols in the enzyme forming reversible thiol mercuration.

1397 Cadmium Alters Heat Shock Protein Pathways in SN56 Cholinergic Neurons, Leading to aβ and Phosphorylated Tau Protein Generation and Cell Death


Cadmium, a neurotoxic environmental compound, produces cognitive disorders, although the mechanism remains unknown. Cadmium induces a more pronounced cell death on cholinergic neurons from basal forebrain (BF), mediated, in part, by increase in Aβ and phosphorylated Tau protein levels, which may explain cadmium effects on learning and memory processes. Cadmium downregulates the expression of heat shock proteins (HSPs) HSP 90, HSP70 and HSP27, and of HSF1, the master regulator of the HSP pathway. This downregulation of HSP expression is observed in BF neurons from our laboratory using non-human primates in which we found dysfunctional but an intact nigrostriatal dopaminergic system suggesting that the neuropathology of chronic Mn exposure is different than that observed in PD. Ongoing studies aim to further characterize the behavioral effects and neuropathology of elevated Mn in SLC39A14-KO male and female mice.

1395 Refining In Vitro Models in Neurotoxicology: Comparing Biological Responses in a Neuronal Cell-Type after Manganese Nanoparticle Exposure

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Manganese (Mn) is an industry-used agent. At levels of overexposure, Mn has been implicated in neurodegenerative complications such as the manifestation or exacerbation of Parkinson-like or Parkinsonian symptoms, i.e. tremor production and gait difficulties. Mn influx is localized in the basal ganglia and is associated with neuronal death of dopaminergic cells in this region. Biological Mn acts as a deoxidizing and desulfurizing agent, creating two biochemical mechanisms for which apoptosis of dopaminergic cells can occur. In the deoxidization pathway, Mn acts as a reducing agent and has implications related to the production of reactive oxygen species and sustained cellular oxidative stress, which often induces apoptosis. In the desulfurization pathway, the high affinity of Mn to bind with sulfur perturbs the normal function of amino acids, such as a cysteine and methionine, inducing apoptosis. In addition, Mn acts as an inhibitor of the enzymes involved in the desulfurization pathway, which interferes with the cerebral spinal fluid. Ongoing in vivo imaging experiments are assessing the effects of Pb on developing dendritic arbors. Collectively, these results reveal new information about how Pb exerts toxic effects on brain development and suggests that Pb-related toxicity may be mediated at least in part by compromised TH physiology in the developing brain.

1396 SLC39A14 Knockout Mice: A Genetic Model to Understand Manganese-Induced Neurotoxicology


Chronic exposure to high levels of manganese (Mn) results in a debilitating form of parkinsonism with dystonia and cognitive function deficits in humans and non-human primates. SLC39A14 is now recognized as a Mn influx transporter. Homozygous loss of function genetic mutations in humans result in behavioral manifestations of parkinsonism with dystonia that is not responsive to typical treatment of idiopathic Parkinson’s disease (IPD). The use of the SLC39A14 knock-out (KO) mouse model provides us with a powerful tool to study and describe the neuropathogenesis of chronic Mn exposure along the lifespan. Preliminary characterization of the locomotor behavior of SLC39A14-KO male and female mice show impairment in locomotor behavior as adults relative to age-matched wildtype (WT). Preliminary analysis of striatal dopamine concentrations using HPLC with electrochemical detection indicates no apparent differences between SLC39A14-KO and WT male mice dopamine levels. Similarly, there was no apparent differences in tyrosine hydroxylase immunostaining in the striatum in SLC39A14-KO versus WT adult male mice. Blood Mn concentrations in the SLC39A14-KO adult male mice (941 ± 8.45 µg/L, n=5) were on average nearly 40 times those of WT mice (24.63 ± 5.39 µg/L; n=6) confirming the accumulation of Mn in the blood (p<0.0001). These preliminary results indicate that SLC39A14-KO mice with high blood Mn concentrations express significant locomotor impairment as adults in the apparent absence of a change in dopamine concentrations and tyrosine hydroxylase levels in the striatum. Our preliminary findings suggest that the locomotor deficits resulting from the accumulation of Mn in the SLC39A14-KO male mice are not associated with the loss of nigrostriatal dopaminergic terminals in the striatum. These studies are consistent with previous results from our laboratory using non-human primates in which we found dysfunctional but an intact nigrostriatal dopaminergic system suggesting that the neuropathology of chronic Mn exposure is different than that observed in PD. Ongoing studies aim to further characterize the behavioral effects and neuropathology of elevated Mn in SLC39A14-KO male and female mice.
trast, two additional cysteines in SPR (Cys133 and Cys234) were not modified by MeHg and these amino acids are structural elements of the enzyme and likely inaccessible to MeHg. Taken together our data demonstrate that accessible 3 cysteine residues in SPR are important targets for MeHg. By inhibiting SPR, neurotransmitter biosynthesis is suppressed, a process that can result in MeHg-induced neurotoxicity. Supported by NIH grants AR055073, NS108956, NS079249 and ES005022.

1399 Neurotoxicity of Metal Mixtures Containing Cadmium, Lead, and Manganese in the Nematode Caenorhabditis elegans

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Heavy metals are a class of persistent environmental toxicants and harmful to animals and human health. Cadmium (Cd) and lead (Pb) are two of the most common toxic heavy metals that have been linked with cancers and malfunction of nervous system, while contamination of manganese (Mn) is usually coexisted with Cd and Pb in environmental and occupational settings. Studies are regularly conducted to examine the toxic effects of individual metals, however, potential health and toxic effects of the mixture containing two or more heavy metals are largely unknown. In this study, we investigated toxic effects of single Cd, Pb and Mn as well as their binary and ternary mixtures in the nematode Caenorhabditis (C.) elegans. The toxic outcomes, including effects on growth, reproduction and feeding behavior, were measured using high-throughput platform analysis (COAPS Biosort). The transgenic strain BY250 (Pdat-1::GFP) that expresses GFP in dopaminergic neurons was used to further explore the neurodegenerative effects induced by single metals or their mixtures. The combination index (CI) for mixtures effect was calculated using isobolograms methods. Following exposure to single metals and mixtures (combination concentration at 0 to 4 times of EC50 of each single metal), we found significant toxic effects on growth, reproduction, and feeding behavior in C. elegans. For single metals, the toxicity order for growth, reproduction and feeding was similar, Pb>Cd>Mn. For metal mixtures, the Mn+Cd induced a less-than-additive (antagonistic) effect in C. elegans whereas the mixture of Cd+Pb, Pb+Mn, and Cd+Pb+Mn induced a more-than-additive (synergistic) effect. About 65% of worms exposed to mixture of Cd+Mn exhibited dopaminergic neurodegenerative toxic lesions, and such neurotoxic effects were found in 85%, 70% and 75% of worms exposed to mixture of Cd+Pb, Mn+Pb and Cd+Pb+Mn, respectively. These results showed combinatory toxic and neurodegenerative effects of heavy metal mixtures, with Cd, Pb, and Mn and future studies will be focused on characterization of dose-response patterns and identification of potential molecular mechanisms in C. elegans model.

1400 Methylmercury In Vivo Preferentially Stimulates Spontaneous GABAergic Synaptic Transmission in Brainstem Hypoglossal Motorneurons (MNs) of Mouse

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Amyotrophic lateral sclerosis (ALS) is a progressive, degenerative, and fatal neurological disorder characterized by decreased skeletal muscle function due to loss of upper and/or lower motor neurons (MNs). The exact cause of ALS remains elusive. Gene-environment interactions likely contribute to the etiology of ALS. We previously showed that chronic exposure of mice overexpressing the human superoxide dismutase 1 (hSOD1) gene mutation (hSOD1<sup>10</sup>); G93A) mouse model for ALS, to methylmercury (MeHg), accelerates the onset of ALS-like phenotype in hSOD1 mice. We also showed that in vivo MeHg exposure preferentially potentiates AMPA-mediated currents, while simultaneously reducing GABA-mediated currents in brainstem hypoglossal MNs from G93A mice. In those studies, both AMPA- and GABA-mediated currents were evoked by direct applications of AMPA and GABA onto the postsynaptic hypoglossal MNs. Thus, the MeHg-induced effects on these currents appear to be a postsynaptic mechanism of MeHg actions. To test if in vivo MeHg exposure also acts presynaptically to affect synaptic function, we examined changes in spontaneous glutamatergic and GABAergic synaptic transmission in hypoglossal MNs in brainstem slices prepared from G93A, hSOD1<sup>10</sup>; G93A mice and WT mice following in vivo exposure to MeHg for defined time periods. Daily exposure of mice to 3 ppm MeHg via drinking water began at postnatal day 28 (PND28). At PND47, 64 and 84, fresh brainstem slices were prepared and spontaneous excitatory or inhibitory postsynaptic currents (eSPSCs or iSPSCs) were examined using whole cell recording techniques. Untreated littermates were sacrificed at the same time points to serve as control. MeHg exposure appeared to potentiate spontaneous release of GABA expressed as an increase in iSPSC frequency but had no significant effect on eSPSCs in hypoglossal MNs. Examination of the action potential firing pattern also revealed no significant difference between control and MeHg-treated mice. Thus, consistent with our previous in vitro studies, chronic in vivo treatment with MeHg appears to preferentially affect GABAergic systems. Supported by NIH grants ES024064.

1401 Huntingtin and Cadmium Induce Striatal Neurotoxicity and Neurodegeneration via Altered Metal Transport and Protein Kinase C Dependent Oxidative Stress and Apoptosis Signaling Mechanisms

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Huntington’s disease (HD) is functionally linked to environmental factors including cigarette use and dyshomeostasis in the levels of metals. 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 huntingtin (HTT), responsible for the majority of cases of HD in patients, in combination with Cd exposure would cause neurotoxicity and neurodegeneration via increased intracellular accumulation of Cd and activation of oxidative stress signaling mechanisms in a mouse striatal cell line model of HD. We report that heterozygous HTT striatal cells are significantly more susceptible to Cd-induced cytotoxicity as compared to wild-type HTT cells upon exposure for 48 h. The heterozygous HTT and Cd-induced cytotoxicity led to a NADPH oxidase (NOX) mediated oxidative stress that was attenuated by exogenous antioxidants and a NOX inhibitor, apocynin. Heterozygous HTT coupled with Cd exposure caused increased expression of protein kinase C ζ (PKCζ) and other key oxidative stress proteins levels, enhanced the activation of caspase-9 and caspase-3 mediated apoptosis, and blocked the overexpression of extracellular signal-regulated kinase (ERK). We observed significantly greater intracellular accumulation of Cd and reduced expression of divalent metal transporter 1 (DMT1) protein in the heterozygous HTT striatal cells upon Cd exposure. Treatment with zinc, manganese, and iron as well as exogenous antioxidants significantly attenuated the Cd-induced cytotoxicity. Collectively, these results demonstrate that heterozygous HTT exhibits greater neurotoxic properties when coupled with Cd exposure to cause cell death via caspase mediated apoptosis, altered metal transport, and modulation of ERK and PKCζ dependent oxidative signaling mechanisms.

1402 Role of Internal Calcium Pools during Acute Methylmercury-Mediated Increase in Internal Calcium Concentration in C57BL6J Mouse Spinal Cord Slices

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Methylmercury (MeHg) is an environmental organic contaminant of current concern. In isolated motor neurons in culture, a key contributor to MeHg neurotoxicity is dysregulation of intracellular calcium (Ca<sup>2+</sup>), homeostasis and subsequent hyperexcitability. The MeHg-mediated increase in Ca<sup>2+</sup> concentration (Ca<sup>2+</sup><sub>calc</sub>) occurs in two kinetically distinct phases. Phase 1 is due to Ca<sup>2+</sup> release from the cytosolic Ca<sup>2+</sup> pools: mitochondria and smooth endoplasmic reticulum (SER). Phase 2 corresponds to Ca<sup>2+</sup> entry into the cell across the plasma membrane. The relative contributions that the mitochondria and SER have to MeHg-induced increases in motor neuron (Ca<sup>2+</sup>), have not yet been reported. The aim of this project is to elucidate the role of Ca<sup>2+</sup> pools to elevations of Ca<sup>2+</sup> following an acute 20μM MeHg exposure. Adult C57BL6J mice spinal lumbar slices were exposed for 15min to MeHg through continuous superfusion. To record Ca<sup>2+</sup> changes in motor neurons we used Fluo4-AM, a fluorescent Ca<sup>2+</sup> indicator. Data were collected at 15min and 1hr-post MeHg, alone or in the presence of cyanobyl cyanide m-chlorophenyl hydrazone (CCK), and thapsigargin (THP), which uncouple mitochondrial oxidative phosphorylation, and block Ca<sup>2+</sup> uptake through the endoplasmic reticulum Ca<sup>2+</sup>-ATPase (SERCA), respectively. CCK causes release of mitochondrial Ca<sup>2+</sup> by depolarizing the mitochondrial inner membrane potential. We hypothesize that these agents will increase MeHg-mediated Ca<sup>2+</sup> increase, compared to MeHg treatment alone, especially during Phase 1, which depends on Ca<sup>2+</sup> stores. MeHg alone significantly increased Fluo4 fluorescence from baseline at both 15min MeHg (50%) and 1hr-post MeHg exposure (56%). MeHg+THP+CCK treatment significantly increased fluorescence from baseline at 1min (68%) followed by a significant decrease at 1hr-post MeHg (76%). MeHg+THP+CCK treatment significantly decreased Ca<sup>2+</sup> fluorescence at 1hr-post MeHg.
1403 Early Postnatal Manganese Exposure Causes Lasting Changes in Prefrontal Cortex Catecholaminergic Systems Accompanied by Arousal Dysregulation and Heightened Behavioral Reactivity

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Recent animal studies from our group have shown that developmental postnatal Mn exposure causes lasting deficits in selective attention, impulse control, and arousal regulation, recapitulating analogous findings from epidemiological studies in children. Given the critical role that the prefrontal cortex (PFC) catecholaminergic systems play in regulating these functions, we determined the effect of early postnatal Mn exposure (0, 25, or 50 mg Mn/kg/day orally PND 1-21 or lifelong) on evoked neurotransmitter release in the PFC, dentritic spine density on pyramidal PFC neurons, PFC catecholaminergic system protein levels, and behavioral reactivity in the open field (OF; 5 days). Postnatal Mn exposure caused increased OF activity in each of the 5 daily sessions, but only over the first 5 min of each session, indicating deficits in arousal regulation and heightened behavioral reactivity. Mn also reduced the evoked release of norepinephrine (NE) in the PFC, and caused lasting changes in the levels of multiple DA/NE synaptic proteins. These changes included decreased tyrosine hydroxylase, DA and NE transporters, and D1 receptor protein levels, along with increased D2 receptor levels. However, PFC alpha-2A adrenergic receptor levels and dentritic spine density on pyramidal neurons were unchanged by Mn. In light of the possibility of mitochondrial neuroinflammation in altering PFC catecholaminergic systems, we assessed gial fibrillary acidic protein (GFAP), complement C3, and S100A10 protein levels as markers of reactive astrocyes expressing A1 pro-inflammatory (C3) or A2 anti-inflammatory (S100A10) phenotypes. Results show that Mn caused a lasting increase in A2 astrocytes (based on GFAP colocalization analyses), which may contribute to Pb-induced amyloid plaques in brain. The implication on Aβ accumulations in the brain and blood vessel endothelium following Pb exposure. Quantifying fluorescent signals revealed the hypothesis that Pb exposure altered RAGE’s expression and function in the CP, contributing to Pb-induced amyloidal aggregation. Freshly dissected CP, adopting perivascular and intravascular structures using custom in-house developed software. Data showed that Pb exposure increased permeability surface area product (PS), indicating that Pb disrupted the mouse blood-brain barrier system. It is agreed with our hypothesis that Pb exposure at toxic levels would directly damage the brain and induce dementia by allowing blood contents to escape into the brain parenchyma. The implication on Aβ accumulations in the brain and blood vessel affected by various doses of Pb is currently under investigation. Supported by NIH/NIEHS R01 ES027078.

1404 Autophagy: An Early Pathogenic Target of Manganese but a Therapeutic Target of Drp1 Inhibition

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The precise mechanism of manganese (Mn) inducing parkinsonism is not known. At relatively high concentrations, however, this heavy metal has been well reported to induce mitochondrial dysfunction and more recently, mitochondrial fission and autophagic impairment. Given the complex relationship between mitochondria and autophagy, it is not fully understood whether mitochondrial dysfunction precedes impaired autophagy or vice versa. Furthermore, it is not clear if these impairments are the cause or consequence of cytoxicity. To this end, we first performed dose-response studies to establish the LC50 values for multiple cell lines after 24h of Mn treatment: The human M17 dopaminergic neuronal cells (21.7 ± 4.4 μM), the N27 rat dopaminergic neuronal cells (266 ± 7.2 μM) and HeLa cells (32.3 ± 8.7 μM). Next, we deter- mined the effect of Mn on autophagy flux using the autophagy reporter HeLa cells stably overexpressing mRFP-GFP-LC3. Mn, at 62.5 μM, severely impaired autophagy flux. In M17 and N27 neuronal cells, autophagy was also blocked by 125 μM Mn as evidenced by a statistically significant increase in LC3 III and P62 puncta. Using transgenic mice with the same autophagy reporter system as our stable HeLa cells, we injected Mn (50mg/kg, s.c) every 3 days (0, 3, 6) for 3 doses, killed on day 7 and then quantified for autophagosomes and autolysosomes in the brain. Consistent with the in vitro data, Mn treatment significantly impaired autophagy flux in these animals. Interestingly, genetically knocking down dynamin related protein 1 (Drp1), which is commonly known as a mitochondrial fission protein, significantly attenuated autophagy blockade induced by Mn in all of our 3 cell types. To investigate the possibility that Mn impaired autophagy via mitochondrial dysfunction, we assessed autophagy using the Seahorse XFe96 Extracellular Flux Analyzer. mitochondrial membrane potential and mitochondrial morphology in the Mn-treated cell models as described above. None of these mitochondrial parameters were altered. In summary, our results indicate that Mn is capable of blocking autophagy at an environmentally relevant concentration that does not induce mitochondrial dysfunction and cytotoxicity in both neuronal and non-neuronal cells. The discovery that the pathogenic and protective mech- anisms of Mn and Drp1 inhibition, respectively, intersect at the autophagic pathway is novel and suggestive of a therapeutic target.

1405 Pb-Induced Neurotoxicology of the Brain Barrier System: New Implications

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Lead (Pb) is an environmental factor has been suspected of contributing to the dementia including Alzheimer’s disease (AD). Our previous study has shown that Pb exposure at the subtoxic dose increased brain levels of beta-amyloid (Aβ) and amyloid plaques, a pathological hallmark for AD, in APP transgenic mice possibly by inhibiting Aβ clearance in the blood-CSF barrier (BCB). However, it remains unclear how toxic levels of Pb affect Aβ clearance in the blood-brain barrier system. This study was designed to investi- gate whether chronic exposure of toxic Pb affected the permeability of the blood-brain barrier system by using the Dynamic contrast-enhanced CT (DCE-CT) method. To test Pb toxicity in mice, 10 Tg-SwDi mice were orally treated with Pb-acetate at 100 mg/kg (equivalent to Pb at 54 mg/kg) for 4 weeks. After 2-week treatments, 20% of mice died and 60% lost more than 20% of their initial weight. Thus, for the DCE-CT study, 20 Tg-SwDi mice were administrated with Pb-acetate or the equivalent amount of Na-acetate for 1 week from the DET-CT was performed on a Phillips Brilliance CT scanner. The images were acquired at 1201sec, 50ksec, and 1010sec acquisitions were acquired. At the 5sec frame, each subject was infused with 0.2ml of Isovone-370 at 3ml/min, followed by a saline flush. Images were registered across subjects and time. Whole brain regions were segmented using Analyze 12.0 (AnalyzeDirect). To perform morphological modeling, custom written software was designed based on dif- ference images between baseline and peak enhancement. To extract kinetic parameters such as tissue blood perfusion and permeability-surface area, a voxel-wise tracer kinetic modeling was performed and mapped to anatomical structures using custom in-house developed software. Data showed that Pb exposure increased permeability surface area product (PS), indicating that Pb disrupted the mouse blood-brain barrier system. It is agreed with our hypo- thesis that Pb exposure at toxic levels would directly damage the brain and induce dementia by allowing blood contents to escape into the brain paren- chyma. The implication on Aβ accumulations in the brain and blood vessel affected by various doses of Pb is currently under investigation. Supported by NIH/NIEHS R01 ES027078.
increased 10.6% and 107% in respected dose groups at 8 weeks. Quantification of Aβ by ELISA displayed a trend of increased Aβ levels in the CSF, albeit not statistically significant as compared to controls. Taken together, our results suggest that RAGE plays a notable role in mobilizing Aβ between two distinct fluid compartments, i.e., the CSF and blood. Pb exposure, either in vitro or in vivo, appears to stimulate RAGE intracellular trafficking. The implication of these findings on Aβ accumulation in the CSF is currently under investigation. Supported by NIH/NIEHS R01 ES027078.

1407 Whole-Brain Approaches for Investigating Iron Accumulation R2* Show No Excess from Occupational Exposure to Welding Fumes

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Iron (Fe) is commonly found in elevated quantities in the human brain affected by neurodegenerative diseases. While it is unknown how Fe plays in the etiologies of these diseases, welders inhale large quantities of metal particulates in welding fume, including iron (Fe) and manganese (Mn). Mn is a neurotoxin that has been shown to accumulate in the brains of welding fume exposed workers by increasing the magnetic resonance imaging (MRI) R1 contrast. R1 and R2* are MRI parameters that are proportional to Mn and Fe accumulation, respectively. Measurements of Mn in the brain could be confounded by accumulated Fe, mostly altering R2* contrast, but also R1 contrast to some degree. Therefore, monitoring human Fe amount using human quantitative imaging in welders is of consequential interest. While some groups, including our own, have reported increased R2* levels in region-of-interest (ROI) based analyses, such findings were inconsistent and targeted few brain regions. To enable an unbiased whole-brain analysis of Fe accumulation in the brain using MRI, 47 welders and 38 controls were recruited from a local manufacturer. Whole-brain R2* maps were coregistered with T1-weighted structural images using SPM12 and then segmented into 192 different brain regions using Freesurfer. R2* in these segmented ROIs within the brain (e.g. white matter tracts and basal ganglia nuclei) were separately averaged and compared between welders and controls. Student’s t-tests showed no statistically significant differences between controls and welders. Therefore, a more comprehensive analysis using machine learning was used to determine if any patterns using all 192 regions could discriminate between controls and welders. Principle component analysis (PCA) was performed on five different statistics of R2* distributions in each ROI: mean, median, skew, 90th percentile, and maximum value. For example, PCA performed on R2* mean showed that only 32 principle components (PCs) were required to explain 90% of variation in mean within all 192 ROIs. A support vector machine (SVM) with a linear kernel was employed using these 32 PCs but could not distinguish between welders or controls better than chance. Similar results were found for the other four statistics. These null results suggest that R2*, and thus brain Fe accumulation, cannot distinguish welders from controls. This provides some evidence that measures of Mn accumulation shown in previous work in the same cohort is only caused by elevated Mn brain levels and not confounded by elevated iron levels.

1408 LRP-1 Expressions and Distribution across BBB and BCB Following Subchronic Lead Exposure

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Lead (Pb) exposure has been shown to facilitate amyloid plaque formation in transgenic AD mouse models. Transport of β-amyloid peptide (Aβ) between the blood and brain parenchyma is regulated by the receptor for advanced glycation end products (RAGE) for Aβ influx and the low-density lipoprotein receptor-related protein-1 (LRP1) for Aβ eflux in the blood-brain barrier (BBB); however, how these transporters may operate in the blood-CSF barrier (BCB) in the choroid plexus (CP) is unclear. The purpose of this study was to determine whether subchronic Pb exposure affected the expression and subcellular location of LRP1 in the BBB and BCB. Sprague-Dawley rats received oral gavage of 0, 14 (low), or 27 (high) mg Pb/kg, 5x/wk for 4 or 8 weeks. At the end of exposure, brain tissues were dissected, and a “capsular depletion” method was employed to separate cerebral capillary fraction from brain parenchyma. Western blot analysis of capsular and parenchymal frac-
tions demonstrated that LRP1 expression in the hippocampal parenchyma, following low- and high-dose Pb exposure, increased by 34% and 106% of the control, respectively (p<0.05). In the capillary of hippocampus, LRP1 in low- and high-dose groups reduced by 6% and 5% of the control, respectively, although this decreased LRP1 expression did not reach the statistical significance. Confocal imaging studies of the choroid plexus showed that the green fluorescent signals representing LRP1 were relatively evenly distributed throughout cytoplasm in all groups including control, low-dose, and high-
dose groups following 4-week Pb exposure. After 8-week gavage with Pb, the translocation of LRP1 was visibly observed in the choroidal epithelial cells, the LRP1 fluorescent signals moved to the cytosol above the nucleus, which were clinging to the apical membrane. The signal intensities in both low- and high-dose groups were visibly increased as compared with controls. Further quantitation of LRP1 expression in the choroid plexus is currently in progress. Taken together, these results suggest that in vitro Pb exposure does not alter the expression of LRP1 in brain parenchyma, but not in the BBB; Pb exposure also causes the intracellular trafficking of LRP1 in the BCB. The implication of these observations remains unknown. Since LRP1 participates in Ab efflux, the significance of Pb interaction with LRP1 deserves further investigation. Supported by NIH/NIEHS R01 ES027078.

1409 Transcription Factor REST/NRSF Is a Positive Regulator of Tyrosine Hydroxylase and Protects Dopaminergic Neurons against Manganese-Induced Neurotoxicity

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RE1-silencing transcription factor/neuron-restrictive silencer factor (REST/ NRSF) is a transcriptional factor which is originally discovered to silence neuronal genes in non-neuronal cells. Recent emerging evidence reveals that REST induces neuroprotection against Alzheimer’s disease (AD) and Parkinson’s disease (PD) by silencing human growth hormone (GH) transgene in neuronal cells. However, the effects of REST on manganese (Mn)-induced neurotoxicity have not been tested. In the present study, we investigated if REST can protect Mn-induced neurotoxicity in dopaminergic neurons. Our findings reveal that overexpression of REST attenuated Mn-induced oxidative stress by using CM-H2DCFDA as an indicator for reactive oxygen species (ROS), as well as Mn-induced impairment of mitochondrial membrane potential in mouse catecholaminergic/dopaminergic (CAD) neuronal cells. REST also attenuated Mn-induced TNF-α production by qPCR and ELISA in CAD neurons. REST inhibited Mn-induced levels of proapoptotic proteins Bax and Daxx, while it reversed Mn-decreased antiapoptotic proteins, Bcl-2 and Bcl-xL. Flow cytometry data also showed that REST attenuated Mn-induced apoptotic in CAD neurons by Annexin V/PI staining. Intriguingly, REST, which is known to be a repressor of many genes, served as a positive transcription factor of tyrosine hydroxylase (TH), a dopaminergic neuronal marker, by its binding to RE1/NRSE consensus sites in the TH promoter, and increasing TH promoter activity, mRNA and protein levels in CAD as well as LÜHMES cells (human dopaminergic neuronal cells). REST thus reversed Mn-induced repression of TH promoter activity, mRNA and protein levels in dopaminergic neurons. Taken together, our findings indicate that REST can be a potential molecular target to treat Mn-induced neurotoxicity as well as other neurological disorders associated with dopaminergic neurodegeneration such as PD.

1410 Manganese Exposure Induces Misregulation of Tyrosine Hydroxylase and Dopamine Receptor D2 Protein Expression in Differentiated SH-SY5Y Cells: Implications for an Epigenetic Mechanism of Mn Neurotoxicity

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Epidemiological studies have reported associations between elevated manganese (Mn) exposure during late gestation and early childhood and the development of inattention, impulsivity, hyperactivity, and fine-motor impairments. We developed a rodent model of developmental Mn exposure that recapitulates these behavioral impairments, and found they were accompanied by life-long catecholaminergic system alterations in the prefrontal cortex. Here we sought to develop a neuronal cell model of Mn exposure that demonstrates several of those key findings, including decreased tyrosine hydroxylase (TH) and increased dopamine receptor D2 (D2) protein levels, to elucidate the molecular mechanism(s) underlying developmental Mn neurotoxicity. We hypothesize that Mn induces mis-regulation of TH and D2 gene expression via an epigenetic mechanism. To test this, SH-SY5Y neuroblastoma cells were exposed to 0, 100, or 300 μM Mn for 8 days while undergoing retinoic acid and TPA-induced catecholaminergic differentiation. Cell differentiation into a neuronal phenotype was verified via cell morphology and increased synaptophysin expression. The Mn exposure regimen did not cause overt cytotoxicity, based on MITT assay and trypan blue cell counts. TH and D2 protein and gene transcript levels were determined using quantitative immuno- fluorescence microscopy and qPCR, respectively, while DNMT enzyme
activity and protein levels were determined using a kinetic enzyme assay and immunofluorescence microscopy, respectively. TH and D2 promoter methylation state is being determined by bisulfite sequencing. Mn exposure decreased TH and increased D2 protein levels, consistent with results from our animal model. We expect reduced DNMT enzyme activity and altered TH and D2 gene transcripts and methylation status to corroborate our observed protein level changes (analysis ongoing). Understanding whether Mn causes lasting changes in expression levels of catecholaminergic system proteins via an epigenetic mechanism will fill an important knowledge gap on the mechanisms of developmental Mn neurotoxicity. These findings will also elucidate how Mn causes the behavioral impairments in animal models and children, and potentially provide molecular targets to develop preventative therapies.

**1411 Role of Akt/AMPKa-Regulated Autophagy Pathway in Arsenic-Induced Neurotoxic Injuries**

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Inorganic arsenic (iAs) is a common toxic heavy metal and carcinogenic factor and is easily detected in pressurized groundwater veins, environmental pollutants and industrial waste. Recently, epidemiological studies have suggested a possible relationship between iAs exposure and neurodegenerative disease risk level changes. However, inorganic arsenic (iAs) exposure has been demonstrated to cause neuro-pathological effect in neuronal cell remain unclear. In this study, the results show that iAs exposure reduces the level of cell viability and apoptotic events (such as caspase 3, caspase 7, PARP) and upregulated Akt and AMPKα phosphorylation as well as autophasosome formation (acidic vesicular organelles, AVOs) and autophagy (such as atg 5, atg 12, atg 10). Inhibition of activation of Akt by LY294002 and AMPKα by compound c potentiated iAs-induced apoptosis and alleviated iAs-induced autophagy. Taken together, these results suggest that iAs-induced neurotoxicity is reduced through an activation of Akt/AMPKα-regulated pathway pathway.

**1412 Lead (Pb) Exposure Induces Dopaminergic Neurotoxicity in Caenorhabditis elegans**

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Oxidative stress is linked to many pathological conditions including the loss of dopaminergic neurons in Parkinson’s disease. Lead (Pb) is a ubiquitous pollutant and studies have shown that Pb cause dopaminergic dysfunction, however, the mechanism remains unexplored. Hence, this study sought to investigate the effect of Pb exposure on dopaminergic neurodegeneration and function as well as the expression level of some dopaminergic signalling and oxidative stress genes in Caenorhabditis elegans. Pb treatment result in gradual loss of dopaminergic cell morphology and structure in worms expressing green fluorescent protein (GFP) under a DAergic cell specific promoter. HPLC analysis revealed a significant decrease in dopamine content in worms treated with Pb when compared with control. In addition, the expression levels of dat-1, sod-1, gst-4 and skn-1 genes were altered in Pb treatment. In summary, our results revealed that Pb exposure induces dopaminergic dysfunction in C. elegans. This action could be attributed to oxidative stress-induced degeneration as initiated by Pb treatment.

**1413 Role of Internal Calcium Pools during Acute Methylmercury-Induced Cell Death in the C57BL6J Mouse**

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Methylmercury (MeHg) is an environmental neurotoxicant of contemporary concern that causes a cascade of cellular effects leading to cell death. MeHg targets a-motor neurons (oMNs) located in the lumbar region of the spinal cord and eMNs responsible for skeletal movement; their degeneration is a key feature of amyotrophic lateral sclerosis. MeHg-induced cell death results from uncontrolled increase in internal calcium concentration ([Ca\(^{2+}\)]. The initial effect is release of Ca\(^{2+}\) from internal organelles (mitochondria and smooth endoplasmic reticulum (SER)) followed by extracellular Ca\(^{2+}\) entry. Our purpose was to elucidate the immediate and delayed contribution that mitochondria and SER provide during MeHg-induced cell death. This was being performed using lumbar spinal cord slices isolated from C57BL6J mice, and calcine-AM, a fluorophore that label viable cells green. Cell viability was determined following a 15min [20μM] MeHg exposure in the presence and absence of chemicals that modulate internal Ca\(^{2+}\) pools: Carbonyl cyanide m-chlorophenyl hydrzone (CCCP) and thapsigargin. CCCP depolarizes the inner mitochondrial membrane increasing cytosolic Ca\(^{2+}\). Thapsigargin blocks the SER Ca\(^{2+}\)-ATPase (SERCA), preventing uptake of cytosolic Ca\(^{2+}\). We hypothesized that CCCP + thapsigargin treatment would increase [Ca\(^{2+}\)], increasing further incidence of MeHg-induced cell death. MeHg alone significantly decreases viability from baseline at 3hrs post-MeHg by 0.65 relative change. Internal Ca\(^{2+}\) stores contribute to MeHg-induced cell death since the MeHg + CCCP + thapsigargin shows a significant viability loss by 0.52 relative change from MeHg control group. This project sheds light on the role of Ca\(^{2+}\)-organelles to MeHg-induced neuronal death. This research is important because it sheds light on the role of Ca\(^{2+}\)-containing organelles, mitochondria and SER, to MeHg-induced motor neuronal death.

**1414 Neurotoxicity of Low-Concentration Chronic Metal Exposures to Motor Neurons In Vitro**


Amyotrophic lateral sclerosis (ALS) is the most common adult-onset paralytic disorder characterized by the degeneration of motor neurons (MNs). ALS is mainly a sporadic disease but about 10% of patients have a familial history. Most mutations linked to ALS present a variable penetrance and a wide range of age at onset and disease progression, indicating there is more to the disease than merely a genetic component. Environmental factors implicated in ALS are still elusive, but exposure to chronic-low-level metal toxicity has been proposed to contribute to ALS and other neurodegenerative diseases. However, little is currently known about the vulnerability of MNs to long-term and low-level metal exposures. To begin addressing this gap in knowledge, we evaluated the cytotoxicity of manganese (Mn), selenium (Se), lead (Pb), and arsenic (As) on MNs from primary spinal cord cultures. To better model chronic low-level exposures, we exposed the spinal cord cultures for a period of 7 days to metal concentrations ranging from 0.25μM-100μM. Exposure started at day in vitro (DIV) 1, cultures were fixed at DIV 8, then immunostained for the neuronal marker SMI32-immunopositivity and a healthy cell morphology. Our results show that for a 7-day-exposure period the order of potency for the tested metals is: As > Se > Mn > Pb. The lethal concentration of each metal required to kill 50% (LC\(_{50}\)) of the MN cell population is 1, 2, 25, and 70μM respectively. This is the first time that cytotoxicity of each of these metals has been evaluated in a 7-day-exposure paradigm and in an ALS-relevant cell type, such as primary MNs. Currently, we are evaluating the cytotoxicity of the same set of metals in MNs that carry a genetic mutation (G928S in the TDP-43 gene) associated with ALS. By comparing the LC\(_{50}\) obtained in WT versus TDP-43 mutant MNs, we hope to bring insights into potential differences in MN susceptibility to metal toxicity based on gene-environment interaction. Our next step will be to chronically expose WT and TDP-43 mutant mice to metal candidates to provide information on the potential of metal exposure to contribute to the pathology of ALS. Identifying environmental modifiers to ALS is pivotal for the development of treatments and prevention strategies.

**1415 Cytotoxicity of Copper (II) Octanoate on Mouse Hippocampal Astrocytes**

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Copper, an essential trace element, is crucial for various biological processes and mechanisms associated with maintaining homeostasis within an organism. In addition, copper has been utilized as a constituent of various pesticides. Copper (II) octanoate, a copper carboxylic acid complex utilized agriculturally for its fungicidal properties, is applied to a wide range of crops and is suitable for use in certified organic production. Due to limited toxicological data, the objective of this study was to investigate the potential toxicity of copper (II) octanoate in vitro. Astrocytes, in conjunction with pericytes and endothelial cells, comprise the blood-brain-barrier and thus one of the first cells within the nervous system to encounter xenobiotics. Previous studies have also demonstrated the capability of astrocytes to regulate copper homeostasis in the brain. Mouse hippocampal astrocytes were isolated from C57BL6J mice and characterized by ELISA for glial fibrillary acidic protein (GFAP). Copper (II) octanoate was synthesized and characterized by mass spectroscopy and elemental analysis. Astrocytes were treated with 100-1000 μM of copper (II) octanoate for 24 Cell viability was assessed and the LC\(_{50}\) was...
1416 Taurine Improved Motor Coordination in Wistar Rats Co-administered with Chlorpyrifos and Lead Acetate

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Living organisms are constantly exposed to complex mixtures of toxicants. Chlorpyrifos (O,O-diethyl-3,5,6-trichloro-2-pyridyl-phosphorothionate) is an extensively used broad spectrum organophosphate insecticide. Lead (Pb) is a ubiquitous and pernicious heavy metal. Taurine (C2H7NO3S) is a potent antioxidant and neuroprotective conditionally essential amino acid. The aim of the study was to evaluate the effect of taurine on motor coordination in male Wistar rats co-administered with chlorpyrifos and lead acetate. In this study, fifty male Wistar rats were assigned into 5 groups of ten animals each as follows: D5 group (distilled water), SY group (soya oil, 1 ml/kg), TU group (taurine, 50 mg/kg), CP+LA group (chlorpyrifos, 4.25 mg/kg, 1/20 LD50; lead-acetate, 233.25 mg/kg, 1/20 LD50; and TU+CP+LA group (taurine+chlorpyrifos+lead acetate). The treatments were administered to the rats once daily by oral gavage for 16 weeks. The motor coordination was assessed on week 15 with the use of a beam walk-performance task. Additionally, the brain malondialdehyde concentration and activities of brain antioxidant enzymes (catalase, superoxide dismutase and glutathione peroxidase) were assessed following the termination of the study. There was a significant increase (p < 0.01) in the widths of slip off the beam of the rats in the CP+LA group compared to those in the TU+CP+LA group. Moreover, the brain malondialdehyde concentration was increased, while the activities of brain antioxidant enzymes were reduced in the CP+LA group compared to the TU+CP+LA group. However, there were improvements in the motor coordination and activities of brain antioxidant enzymes, as well as attenuation of brain malondialdehyde concentration in the groups treated with taurine. It is surmised that taurine improved the motor coordination of the rats through its antioxidant properties.

1417 Toxicologic Evaluation of SABA-10 (X-7590-15)

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Seeds of Sabadilla, Schoencaulon officinale Grey (Liliaceae) have been used as an insecticide since pre-historic times in Mesoamerica. The insecticidal activity of sabadilla arise from the alkaloid fraction of the seed (3-6%) generally referred to as sabadilla or veratrums alkaloids. Among the alkaloid fraction, two lipophilic alkaloids veratridine and cevadine have the highest reported insecticidal potency. The toxicity of SABA-10 (formulation consisting of 10% two lipophilic alkaloids veratridine and cevadine) was determined as 350 μM. Studies have shown copper-containing compounds to activate the transcription factor Nrf2, a regulator for various antioxidant and detoxification pathways. Fluorescence microscopy of astocytes treated with 350 μM indicated an increase of Nrf2 translocation into the nucleus from the cytosol as compared to untreated cells. This suggests that the toxicity of copper (II) octanoate may be associated with the generation of oxidative stress and the subsequent induction of Nrf2 mediated pathways.

1418 Differential Effects of Organophosphate Insecticides and Their Metabolites on Neuronal Network Activity and Function Assessed in Primary Rat Cortical Cultures Using Microelectrode Array (MEA) Recordings

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Organophosphate (OP) insecticides like chlorpyrifos (CPF) and diazinon (Dz) are widely used in agriculture and household applications. Human exposure occurs mainly in occupational settings as well as via the food chain. Neurotoxicity of OP insecticides is (primarily) exerted via irreversible inhibition of acetylcholine esterase (AChE), leading to neuronal overstimulation and malfunction, and with the -oxon metabolites being more potent than the parent compound. Recently, OP insecticides have been demonstrated to inhibit calcium influx at levels that do not induce inhibition of AChE. Therefore, this study aimed to assess the effects of two widely used OP insecticides and their -oxon metabolites on intracellular calcium homeostasis as well as development and function of spontaneously active neuronal networks in rat primary cortical cultures. Effects of acute exposure to Dz and CPF and their -oxon metabolites on calcium homeostasis were assessed using single-cell imaging of the Ca2+-sensitive dye Fura-2. Considering the pivotal role of intracellular calcium homeostasis in development and function of neuronal networks, effects on spontaneous neuronal activity were assessed using micro-electrode arrays (MEAs) following acute (30min-48h) and chronic (up to DIV 21) exposure. Acute exposure to both the OPs and the metabolites induces a concentration-dependent inhibition of depolarization-evoked calcium influx. Similar chronic exposure revealed a preferential effect for Dz as compared to CPF and their -oxon metabolites. A concentration-dependent inhibition of spontaneous electrical activity. However, chronic developmental exposure results in a concentration-dependent increase of network activity for Dz, whereas CPF affects network activity only at high (>10μM) concentrations. Importantly, for all observed effects, the parent compound showed more pronounced effects. These effects occurred at concentrations below effect concentrations for AChE inhibition, the combined results indicate that non-AChE related mechanisms may play a role in the neurotoxicity of OP insecticides.

1419 Molecular Mechanisms for the Antidepressant Effects of Ketamine in a Rat Model of Gulf War Illness

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Chronic exposure to organophosphate pesticides and nerve gas is thought to underlie Gulf War Illness (GWI) which afflicts ~1 103 of the First Gulf War Veterans. Among other symptoms, GWI is chiefly characterized by therapy-resistant depression. There is strong pre-clinical and anecdotal evidence that Ketamine (KET), an NMDA receptor antagonist, produces a rapid onset and sustained antidepressant effect. This is attractive since existing antidepressants show a delay of therapeutic onset. Here, we investigated antidepressant effect of KET in a rat model of GWI. Male Sprague-Dawley rats (3-m) were exposed to DFP (0.5 mg/kg s.c., 5-d) and 6-m later assessed using the Forced Swim Test (FST). DFP exposed rats exhibited a significantly higher immobility time compared to age-matched control rats. DFP rats treated with KET produced a dose-dependent decrease in immobility time when tested at 1-h. A low, sub-anesthetic KET dose (10 mg/kg, i.p.) produced maximal reduction. The neurotoxicity of OP insecticides and their metabolites on neuronal network activity was assessed using micro-electrode array (MEA) recordings. The studies show that inhibition of the NMDAR-Ca2+ pathway plays a role in the rapid onset antidepressant effects of KET while the sustained effect was calcium-dependent in neuronal networks. The studies showed that at a low, sub-anesthetic dose KET produced maximal reduction.
antidepressant actions involves upregulation of BDNF signaling. Our studies support evidence that KET therapy could provide an important alternative for GWI depression.

1420 Selective Behavioral Deficits Months Following Exposure to Gulf War Illness Chemicals in a Mouse Model: Modulation by the Immunological Therapeutic LNFPIII

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Approximately 30% of veterans from the 1990-1991 Gulf War (GW) suffer from Gulf War Illness (GWI), a complex illness with a number of immunological and neurological symptoms including systemic inflammation. The precise etiology of GWI remains undetermined, but overexposures to GW-related chemicals, including carboxylesterases (CarbEs) and butyrylcholinesterase (BChE), are implicated in it. Currently, there are no effective treatment options for GWI. Here, an established GWI mouse model (10 days of PB/PM exposure) was used to explore (1) the long-term behavioral effects of deployment-related GW chemicals exposure and (2) the ability of a novel immunological treatment, LNFPIII, to modulate the behavioral effects of GWI when given months after the end of initial GW chemicals exposure. A battery of behavioral tests, used for assessment of memory, mood, and motor function in rodents were performed. From the data analyzed to date, motor/sensorimotor function appears to be most affected by GWI as evident by increased steps to complete Gait Test (GT), increased contact and bromide time (PB), and the pesticide permethrin (PM), are implicated in it. Currently, there are no effective treatment options for GWI. Here, an established GWI mouse model (10 days of PB/PM exposure) was used to explore (1) the long-term behavioral effects of deployment-related GW chemicals exposure and (2) the ability of a novel immunological treatment, LNFPIII, to modulate the behavioral effects of GWI when given months after the end of initial GW chemicals exposure.

1421 Butyrylcholinesterase Inhibition and Its Effects on Ghrelin-Induced Food Intake

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Organophosphorus pesticides (OPs) elicit acute toxicity by inhibiting acetylcholinesterase. However, they also bind and inhibit additional hydrolases including carboxylesterases (CarbEs) and butyrylcholinesterase (BChE). Recent studies demonstrated that BChE can inactivate the peptide hormone ghrelin to its des-acyl form via des-acytylation. Ghrelin is a peripherally-produced hormone which acts on the central growth hormone secretagogue receptor (GHSR) to orchestrate orexigenic effects. We hypothesized that exposure to a BChE-selective cholinesterase inhibitor (tetraisopropyl pyrophosphoramide, iso-OMPA) would increase the orexigenic effects of acyl-ghrelin by blocking its des-acytation, in the absence of any cholinergic toxicity. Male, Long-Evans rats (150-175 g) were divided into four treatment groups: veh/veh, iso-OMPA/veh, veh/ghrelin, and iso-OMPA/ghrelin (n=7-8/group). Chow and sucrose consumption (using a two-plate choice paradigm; 5% sucrose vs water) were monitored using daily 3-hour sessions for nine days to determine baseline consumption. On day 10, animals were either given vehicle (saline) or iso-OMPA (25 mg/kg, sc; 2 ml/kg). Twenty four hours later they were given either vehicle (saline) or ghrelin (0.2 mg/kg, ip; 1 ml/kg) and 3-hour consumption was measured as before beginning immediately after ghrelin administration. Tissues (plasma, hypothalamus and liver) were then collected for biochemical assays. Iso-OMPA elicited extensive BChE inhibition in plasma (86%), but lesser inhibition in liver (45%) and hypothalamus (58%). Ghrelin-treated rats consumed more chow compared to baseline. Iso-OMPA had no apparent influence on ghrelin-stimulated intake. No differences in sucrose consumption were noted among the four treatment groups. These results suggest that BChE inhibition may not alter the orexigenic effects of ghrelin in rats. The lack of effect of iso-OMPA on chow intake may be due to a number of reasons including insufficient BChE inhibition in liver or other tissues, or the known role of other hydrolases in acyl-ghrelin metabolism. Future studies may evaluate higher doses of iso-OMPA or alternative des-acyl ghrelin inactivation mechanisms. Supported by the OSU Interdisciplinary Toxicology Program (KHI) and Howard Hughes Medical Institute (KM).

1422 Chronic Low-Dose Diazinon Exposure of Rats during Gestation Causes Long-Term Neurobehavioral Effects Lasting into Adulthood

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Diazinon, a widely used organophosphate pesticide, can with pulsatile exposure during development cause long-term neurobehavioural impairment. The current study investigated persisting effects of chronic low-level infusion of 0, 0.5 and 1.0 mg/kg/day of diazinon throughout gestation via osmotic minipumps (N=10-11 litters per condition). One male and one female offspring from each litter was assessed with a battery of behavioral tests that started at four weeks of age and continued into adulthood. Litter was used as the unit of variance for the analysis of variance test of significance, with sex as a within litter factor. Chronic diazinon exposure from pre-mating until the postnatal period caused a significance of hyperactivity as assessed in the Ankle-8 apparatus. Dose comparisons detected significant (p<0.05) locomotor hyperactivity in both the 0.5 and 1.0 mg/kg/day diazinon dose groups. Diazinon exposure also caused a significant impairment in novel object recognition. This higher diazinon dose (1 mg/kg) caused a significant impairment in the novel object recognition task when the male and female offspring were tested during adolescence. A significant (p<0.05) impairment in preference for the novel object was seen with diazinon. The 1 mg/kg diazinon dose (p<0.05) showed significantly less preference for the novel object than controls during the first five minutes of the novel object recognition test. This effect on cognitive function did not appear to be the result from a generalized dysfunction as the rats were not found to differ from controls on tests of emotional function. This study is continuing with additional cohorts to determine the reliability of the effect. These behavioral tests and companion neurochemical assessment will be used to guide future tests of drug treatment to alleviate the persistent behavioral impairment due to prenatal exposure to diazinon. Supported by the Duke University Superfund Research Center (ES010356).

1423 Microglia-Specific Knockout of NF-kappaB/IKK2 Protects against Neurotoxic Injury from Exposure to Rotenone

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A number of environmental pesticides have been implicated as risk factors for Parkinson’s disease (PD) due their capacity to damage dopaminergic neurons. Rotenone is a naturally occurring insecticide that potently inhibits mitochondrial complex I, leading to neurochemical and neuropathological deficits that closely resemble those in idiopathic PD, including loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), decreased dopamine levels and aggregation of alpha-synuclein. Although rotenone exposure causes activation of microglia and astrocytes that is associated with the progression of neuronal injury, whether neuroinflammation precedes the majority of injury to dopaminergic neurons is not known. Neuroinflammatory activation of microglia and astrocytes is highly regulated through the transcription factor, nuclear factor-kappaB (NF-κB). We therefore postulated that gene ablation of IKK2 in microglia would be neuroprotective against rotenone by preventing inflammatory injury to dopamine neurons. We therefore generated microglia-specific KO mice for IKK2 by crossing Cx3cr1-Cre mice with iK2-flox mice and exposed homozygous progeny (or iK2flox genotype controls) to 2.5 mg/Kg/day rotenone for 14 days. IKK2 KO mice had dramatically decreased expression of IKK2 in microglia and did not show induction of the NLRP3 inflammasome following exposure to the Toll Receptor 4 (TLR4) agonist, lipopolysaccharide (LPS). Wildtype mice exposed to rotenone showed loss of tyrosine hydroxylase-positive dopamine neurons in the SNpc, as well as pronounced activation of microglia and astrocytes, that was prevented in microglia-specific IKK2 KO mice. Rotenone-induced locomotor deficits were also largely reversed in IKK2 KO mice. These data demonstrate that inflammatory activation of microglia following rotenone exposure is critical to both activation of astrocytes and to neurotoxic injury to dopaminergic neurons.
1424 Adult Exposure to the Pesticide Chlorpyrifos Causes Short-Term Behavioral Effects in the Zebrafish

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Organophosphate pesticides (OP), such as chlorpyrifos (CPF), were introduced as alternatives to organochlorine pesticides such as DDT, which was banned due to toxicity and environmental hazards in the early 1970s. Since then, many American farm workers have been chronically exposed to OP throughout their adult lives. Recent evidence suggests that farm workers chronically exposed to pesticides as young adults face greater risk for adverse cognitive effects and neurodegenerative diseases as they age. Our lab has recently observed that chronic adult exposure to CPF can produce short and long term effects on behavior in the zebrafish, including increased anxiety-like behaviors. The aim of the present study is to demonstrate whether adult exposure to CPF carries similar risks. Naive adult zebrafish (6-8 months of age) were separated into tanks of 10 mixed-sex fish and exposed to CPF concentrations of 0.3, 1 or 3 µM in 0.001% DMSO or to the vehicle alone over the course of two weeks. These doses were selected to fall below the threshold for overt toxicity. This relatively brief exposure models the consequences of chronic CPF exposure during a short period in early-mid adulthood, rather than over a lifetime. Following a 1 week recovery period, the fish were assessed in a behavioral test battery with assays for anxiety-related behavior, sensorimotor response and habitation, social interaction, predator avoidance. Fish were tested again at 14-months of age to assess the persistence of these effects and/or the emergence of aging-related deficits. The relatively brief exposure time of two weeks was shown to dose-dependently impact multiple behavioral outcomes 1-week after exposure, although these effects were not persistent into late adulthood. The highest dose of CPF reduced anxiety-like responding in the novel tank test at 1-week post-exposure and enhanced social approach behaviors in the shoaling test (p<0.05). Similar trends in the middle dose group approached significance (0.05<p<0.06). The low dose of CPF did not produce these effects, but rather impaired the habituation of acoustic startle in the tail test (p<0.05). These data show that adult exposure to CPF-induced neurotoxicity, although the brief exposure used here did not produce effects which persisted into late adulthood. Further testing with longer exposure periods will be necessary. Supported by the Virtual Consortium for Translational/Transdisciplinary Environmental Research (ViCTER) project (R01ES024428-0351).

1425 Inhibition of ER Stress Attenuates Deltamethrin-Induced Hippocampal Neuroinflammation in Mice

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Emerging evidence indicates that endoplasmic reticulum (ER) stress and neuroinflammation contribute to the pathogenesis of neurodegeneration and cognitive dysfunction. Previously, we reported that exposure to pyrethroid insecticide deltamethrin causes hippocampal ER stress, apoptosis, reduction of neurotrophins, and learning deficits in adult mice. Recently, we found that deltamethrin exposure also increases the markers of neuroinflammation. Here, we investigated the potential mechanistic link between ER stress and neuroinflammation following exposure to deltamethrin. We found that single oral exposure to very low dose of deltamethrin (1 mg/kg) caused neuroinflammation as mice exhibited with microglial activation and increased protein levels of TNFα, gp91phox, and iNOS in the hippocampus. These changes were accompanied by induction of ER stress as the protein levels of CHOP and GRP-78 were significantly increased in the hippocampus following exposure to deltamethrin. To determine whether induction of ER stress triggers the inflammatory response, mice were treated with two microperitoneal (i.p.) injections of 1 mg/kg salubrinal (an ER stress inhibitor) 24 h and 30 min before the administration of deltamethrin. Inhibition of ER stress with salubrinal prevented deltamethrin-induced TNFα, gp91phox, and iNOS activation. For further confirmation of these results, we performed an additional experiment with microperitoneal cell line (BV2). We found that salubrinal inhibited ER stress and significantly attenuated the levels of TNFα, gp91phox, and iNOS in BV2 cells. Collectively, these results demonstrate that exposure to deltamethrin leads to ER stress mediated neuroinflammation, which may subsequently contribute to neurodegeneration and neuronal dysfunction in mice. Supported in part by 1R01ES027481-01A1 and NEOMED SURF program.

1426 DDT Increases Neuroinflammation and Microglial Activation: Role of Microglial Sodium Channels

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Alzheimer’s disease (AD) is the most common neurodegenerative disease, characterized by the presence of amyloid beta (Aβ) plaques, neurofibrillary tangles and chronic inflammation. Less than 5% of all AD cases are purely genetic in etiology, suggesting that the environment likely plays a role in the etiology of AD. We have previously reported that serum levels of dichlorodiphenyldichloroethylene (DDE), the metabolite of the pesticide dichlorodiphenyltrichloroethane (DDT), were significantly higher in AD patients compared to age-matched controls. Further, we also found increases in the amyloid pathway, specifically for increased mRNA and protein levels of the amyloid precursor protein (APP) and protein levels of Aβ in wild-type and transgenic mice. As Aβ is associated with neuroinflammation, we investigated the effect of DDT on neuroinflammation in mice and primary microglia. Mice exposed to DDT (3 mg/kg every 3 days) from 9-12 months of age demonstrated significantly increased levels of IL-1β mRNA in the cortex, and increased protein levels of TNFα in cortex, hippocampus and serum. Exposure of primary mouse microglia (PMG) to varying doses of DDT (0.5, 1.0 and 5.0 µM) elicited a ~4-5-fold increase in mRNA levels of IL-1β and TNFα respectively, with similar increases in mRNA levels of IL-6, Nos2 and Tnfα. In addition, we measured ~2-4-fold higher levels of Nos2 and TNFα protein by immunocytocchemistry. Mechanistically, the increase in cytoplasmic mRNA was blocked by the sodium channel antagonist tetrodotoxin (TTX), demonstrating the requirement of DDT interaction with sodium channels. These data indicate that DDT increases neuroinflammation that may be the result of direct actions of DDT on microglia, providing a novel pathway by which DDT exposure may contribute to AD risk. Supported in part by NIH R01ES026057.

1427 Characterization of Organophosphorous Pesticides on Acetylcholinesterase Inhibition Using In Vitro Assays with Xenobiotic Metabolic Capability

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Acetylcholinesterase (AChE) inhibition can have significant impacts on human health. A large number of environmental chemicals such as synthesized drug candidates, food additives, and industrial chemicals have not been tested for AChE inhibition activity. Moreover, some chemicals may need to be metabolically activated to show inhibitory effects. In our study, we have developed and characterized a high-throughput screening method with metabolic capability for identifying AChE inhibitors. An enzyme-based high-throughput assay was developed in the current study by using recombinant human AChE combined with human or rat liver microsomes. AChE activity was measured by two methods, one with colorometric and the other with fluorescent readouts. The enzymatic assay with human microsomes (n = 72) showed good performance with coefficient of variation (CV), signal to background ratio (S/B) and z’ factor of 2.03 ± 0.05, 3.29 ± 0.01 and 0.85 ± 0.02, respectively. The assays with microsomes were characterized by testing a group of well-known AChE inhibitors including parent compounds and their metabolites. Many organophosphorous pesticides (OPs), such as chlorpyrifos, tebuquinifos and chlorothoxyfos, only showed inhibitory effects on AChE in the presence of microsomes, showing that these OPs need to be metabolically activated to inhibit AChE. The sensitivities of the assays +/− microsomes ranged from 58% to 85% depending on the assay format and species of microsomes. These results demonstrate that the enzyme-based assays with or without liver microsomes provide a promising tool for the profiling of AChE inhibitors and to study the metabolism of OPs. Disclaimer: This abstract does not reflect US EPA, US FDA, and NIH policies.

1428 Parkinson’s Disease–Relevant Gene Mutations Render C. elegans More Vulnerable to Mancozeb Fungicide Exposure and Mitochondrial Hyperpolarization

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Mancozeb is one of the most widely-used fungicides in the world. The active ingredient, manganese/zinc ethylene-bis-dithiocarbamate (Mn/Zn EBDC), has been linked to neurodegenerative diseases, such Parkinson’s disease (PD). The
vast majority of PD cases, however, are hypothesized to result from gene-environment interactions, not C. elegans strains: wild type (N2), a human LRRK2 G2019S mutant, and a strain with a park2 (pdr-1) deletion (VC1024). Each strain was treated with various concentrations of Mn/Zn EBDC (3.7 or 37 μM) for three consecutive days, with a proportion of the population assessed for lethality and mitochondrial function after each day. Following day 1 treatment, two-way ANOVA of tetrathymidinodamine ethyl ether (TMBE) accumulation indicated that only the presence of the treatment (DF=2, F = 33.98, ***p < 0.0001) contributed to the observed data variation. Post-hoc analysis showed that both mutant worm strains had statistically significantly less TMBE accumulation (**p < 0.01) than wild type worms. By the day 2, both the treatment (DF=2, F = 10.17, **p = 0.0024) and mutation (DF=2, F = 5.34, **p < 0.006), but not an interaction (DF=4, F = 2.43, p = 0.052), accounted for the variance. Contrary to day 1 data, however, WLZ3 (LRRK G2019S) worms showed a statistically significant increase in TMBE accumulation compared to N2 worms (**p < 0.001). This mutation effect was greatest in worms treated with 37 μM Mn/Zn EBDC. By day 3, the mutation alone accounted for the data variance (DF=2, F = 38.65, ***p < 0.0001). While TMBE accumulation levels for WLZ3 worms was similar to N2 worms, post-hoc analysis showed that WL1024 (pdr-1) worms had a statistically significant increase in TMBE (*****p < 0.001) at all treatment concentrations. To determine if the mitochondrial hyperpolarization suggested by augmented TMBE fluorescence adversely affected mitochondrial function, ATP levels were measured in WLZ3 worms. Results indicated statistically significantly increased ATP in day 1, but not other days, in worms treated with either concentration of Mn/Zn EBDC (**p<0.01). These results suggest that cells may compensate for the increased proton gradient by producing additional ATP. Taken together, these data support the hypothesis that subacute mancozeb exposure at levels below those used by agricultural workers leads to mitochondrial dysfunction, a characteristic of PD, and that this dysregulation may be more pronounced in C. elegans with PD-relevant mutations.

1429 Behavioral and Electrophysiological Effects of Early-Life Exposure to the Pyrethroid Insecticide Deltamethrin


Epidemiological data showed a correlation between pyrethroid metabolites in urine and increased risk of ADHD diagnosis in children. In rats, exposure to a commonly used insecticide deltamethrin (DM) 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. Here, we investigate MSNs dysfunction due to developmental DM exposure and aberrations in a battery of behavioral assays. For our early-life exposure model, pregnant female B6 mice were exposed to 3.0 mg/kg of DM throughout pregnancy and lactation. Then, male mice litter-mates from post-natal day ~30 were used for subsequent experiments. We employed whole-cell patch-clamp electrophysiology in coronal brain slices to monitor changes in NAC MSNs firing due to developmental DM exposure. A decrease in the total number of action potentials and instantaneous firing frequency was observed (n=7-12, p<0.05). We are utilizing the same early-life exposure model to conduct a battery of behavioral assays measuring changes in anxiety and depressive like behaviors.

1430 Dithiocarbamate Fungicide Mancozeb Disrupts the Pituitary-Thyroid Axis and Cell Proliferation in Rat Hippocampus

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Mancozeb is extensively used world-wide as a pesticide with millions of pounds applied annually in the United States alone to control plant diseases in the field and during storage. The presence of Mancozeb and its metabolite ethyleneurethane (ETU) in the environment can lead to ingestion, inhalation and/or transdermal absorption. Mancozeb is known to inhibit the enzyme thyroid peroxidase. Based on data obtained from both adult and fetal rats, adult onset hypothyroidism in humans is associated with cognitive dysfunction and depressed mood. 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. This study elucidated the thyroid disrupting potential of Mancozeb and its effects on the modulation of cell proliferation in the hippocampus of rats. Long-Evans rats were exposed to 0, 50, 100, or 150 mg/kg body weight of Mancozeb for 28 days. All animals received intraperitoneal injections of mitotic marker 5-bromo-2-deoxyuridine (BrdU, 50 mg/kg) twice daily on the last 5 days of Mancozeb exposure and were euthanized 24 hours after the last BrdU injection. At the end of exposure, total serum thyroxine (T4) levels were measured. Whole brains were isolated and cryopreserved for sectioning. Mancozeb exposure significantly decreased total T4 serum levels in a dose-dependent manner. Thyroid hormone disruption was reflected in a significant increase in serum levels of thyroid stimulation hormone (TSH) in rats exposed to Mancozeb, indicating a normal negative feedback response of the hypothalamic-pituitary-thyroid (HPT) axis due to low serum T4 levels. The effect of the hypothryoid status on cell proliferation in the hippocampus was assessed using the mitotic marker BrdU used to label dividing cells. A decrease in the number of BrdU-positive cells in the hilar region of dentate gyrus was observed in response to Mancozeb-induced hypothyroidism when compared to the control group. These results indicate that Mancozeb-induced reduction in thyroid hormone disrupts cell proliferation in the hippocampus, which warrants future investigation to address the functional significance.

1431 Antimycobacterial Screening and Safety Evaluation of Tithonia rotundifolia (Asteraceae), an Alien Invasive Plant Species in Southern Africa


Tuberculosis remains a global threat and one of the leading causes of mortality. The emergence of Multidrug-Resistant (MDR) and Extensively-Drug Resistant (XDR) tuberculosis has become a major challenge, especially in Africa. Considering this, the use of medicinal plants as a source of treatment is advocated. Tithonia rotundifolia, a known invasive plant with negative impacts in southern Africa, was screened against non-pathogenic Mycobacterium aurum, M. fortuitum, M. smegmatis and pathogenic M. bovis and M. tuberculosis H37Rv. Antimycobacterial activity of aceton, dichloromethane and hot water extracts of the weed plant was determined using a serial microdilution assay. Cytotoxicity test of the extracts against African Vero monkey kidney, human colon Caco-2 and human liver C3A cells was carried out using the tetrazolium-based colorimetric assay while genotoxicity test was conducted against Salmonella strains TA98 and TA100 using the Ames test. The tested extracts inhibited growth of pathogenic and non-pathogenic Mycobacterium strains, but better activity was displayed against the non-pathogenic strains M. aurum, M. fortuitum and M. smegmatis with minimum inhibitory concentration values ranging between 0.04 and 0.08 mg/ml. No toxicity of the extracts was recorded against the cells tested with the Vero cells showing LC50 values between 0.78 and 0.96 mg/ml and selectivity index (SI) value (LC50/MIC) ranging from 1.05 to 24.05. LC50 values for Caco-2 cells were between 0.198 and 0.32 mg/ml with SI values ranging from 1.01 to 5.83 and LC50 for C3A cells between 0.67 and 0.88 mg/ml and SI value ranging from 2.34 to 18.32. No genotoxic effects were displayed against the Salmonella strains TA98 and TA100. Identification of possible compounds responsible for the observed activity is ongoing. The results from this study serve as a lead for further investigation of this plant for the possible development of effective antimycobacterial treatments.

1432 Effects of Non-Gaseous Oxygen Therapeutic Drug on Five Human Cancer Cell Lines

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According to the National Cancer Institute, in 2018, an estimated 1,733,350 new cases of cancer will be diagnosed in the United States and 609,640 people will die from the disease. Given that cancer cells thrive in hypoxic environments, introducing oxygen into the cancerous environment through oxygen therapy may be vital to the patient, as supplying oxygen to the cells may starve them of the fuel they need to survive. Traditional oxygen therapy is most commonly known as Hyperbaric Oxygen Therapy (HBOT). This method starves them of the fuel they need to survive. Traditional oxygen therapy may be vital to the patient, as supplying oxygen to the cells may allow them to thrive and grow. Newer forms of oxygen therapy such as Hyperbaric Oxygen Therapy (HBOT) have been used to treat certain conditions, but their effectiveness remains uncertain. According to the National Cancer Institute, in 2018, an estimated 1,733,350 new cases of cancer will be diagnosed in the United States and 609,640 people will die from the disease. Given that cancer cells thrive in hypoxic environments, introducing oxygen into the cancerous environment through oxygen therapy may be vital to the patient, as supplying oxygen to the cells may starve them of the fuel they need to survive. Traditional oxygen therapy is most commonly known as Hyperbaric Oxygen Therapy (HBOT). This method still remains a controversial and is not widely used due to the lack of evidence to support its effectiveness. According to the National Cancer Institute, in 2018, an estimated 1,733,350 new cases of cancer will be diagnosed in the United States and 609,640 people will die from the disease. Given that cancer cells thrive in hypoxic environments, introducing oxygen into the cancerous environment through oxygen therapy may be vital to the patient, as supplying oxygen to the cells may starve them of the fuel they need to survive. Traditional oxygen therapy is most commonly known as Hyperbaric Oxygen Therapy (HBOT). This method still remains a controversial and is not widely used due to the lack of evidence to support its effectiveness.
Evaluation of drug-induced gastrointestinal effects is an important aspect in early drug development for some drug classes. Tools for the in vitro assessment of target cells as well as for investigations of potential mechanisms of toxicity affecting the gastric mucosa are not widely available. We sought to develop additional in vitro methods that may potentially identify affected cells in the stomach mucosa therefore leading to enhanced understanding of the mechanism of the drug toxicity. Attention is paid to establish primary cultures of parietal cells isolated from dog stomach were not successful due to poor viability following a density gradient isolation procedure (Percol). Follow-on experiments utilized an innovative approach derived from dog stomach precision-cut tissue slice methodology. Stomach mucosa was dissected free from the submucosa and slices of the isolated mucosa were prepared using a tissue slicer and cultured in Williams E media. Effects of variables such as the thickness of the slices, incubation duration of the tissue slices, and the role of components of the culture media were evaluated. Stomach slices were harvested at 0 (baseline), 24 and 48 hours after the slicing. Cellular morphology and viability of the cells in the slice were evaluated using histopathology. Results indicated that maximum viability of stomach slices is the highest at baseline, followed by 24 hours of incubation. The stomach slice after 48 hours of incubation showed significant necrosis and could not be used for the study. The addition of 10% FBS into the culture media did not improve the viability of the tissue slice. In situ hybridization to identify the H+/K+ ATPase a subunit of the proton pump allowed specific identification of parietal cells. Our preliminary results indicate that the stomach slice culture technique combined with H&E and in situ hybridization provides a useful model system to monitor changes induced by experimental compounds affecting the cell types of the stomach in vitro for studies up to 24 hours in length.

**1434 High-Throughput Assessment of Cardiotoxicity Using Bioreactor Produced hiPSC-Derived Cardiomyocytes**

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Off-target cardiotoxicity effects are the most common cause of delay in approval or even withdrawal of newly developed drugs. The application of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) in safety pharmacology has opened unique opportunities for studying cardiac pharmacology in a platform and prediction model which is comparable with high-throughput approaches. Importantly, the technology has the potential to improve preclinical testing of drug candidates, and to reduce the time and cost of bringing new drugs to market. Calcium ions play a critical role in the electrophysiology of cardiomyocytes and regulate cardiac contraction. Primary human induced pluripotent stem cell-derived cardiomyocytes (Pluricyte® Cardiomyocytes), which recapitulate a human cardiomyocyte’s contractile and electrophysiological profile, we developed a Ca2+-flux assay to evaluate cardiotoxicity of drugs. To enable robust and scalable production of our cells for HTS applications, we have implemented a bioprocessing pipeline comprising staged -the-art bioreactor technologies. The Ca2+-flux assay was developed on a fully automated platform using FLIPR® Tetra kinetic plate reader in combination with FLIPR® calcium 6 assay kit (Molecular Devices). Pluricyte® Cardiomyocytes culturing steps in 384-well plate including plate coating, cell seeding and medium refreshment were performed in a fully automated manner using Fluent® workstation (Tecan). Using this assay, which was validated for average peak amplitude, beat frequency and peak spacing, we evaluated multiparametric effects of a set of 288 test compounds. The effects of compounds were compared to relevant negative (Nifedipine) and positive inotropic (Bayk 8644) and chronotropic (Isoprenaline) controls. The assay was validated using 32 replicates of each control per 384-well plate, revealing a robust assay window (> 2) at high reproducibility (%CV < 20). Altogether, our results confirm that the validated calcium flux assay is ready for high throughput screening to assess cardiotoxicity of compounds during the early phase of drug discovery. Phenotypic screening based on human models does not only reduce the number of late stage compound failures, it also allows continuation of projects that otherwise would fail. These state-of-the-art technologies hold great promise to get better drugs to patients faster.

**1435 Simultaneous Measurement of Contraction, Voltage and Calcium in hiPSC-CMs for the Detection of Inotropic Effects under Blinded Conditions**


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Novel in vitro models based on human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) provide an unprecedented opportunity for next generation cardiac safety assessment and drug efficacy screenings. While current safety approaches are mainly focused on electrophysiological assessments, the ability to identify effects on contractility remains challenging. Within the CRACK-IT InPulse Challenge, we aim to generate a physiologically-relevant contractility platform using hiPSC-CMs. Here, we present one of the platforms being evaluated within CRACK-IT and aim to simultaneously measure contraction, voltage and calcium in hiPSC-CMs to evaluate inotropic effects of compounds under blinded conditions. 7 out of the 10 test compounds were correctly classified as negative or positive inotropes or “no effect” compounds. Epinephrine, a non-selective adrenergic agonist, induced an increase in calcium peak amplitude, a decrease in calcium transient time to peak and a decrease in contraction time (positive inotropic effect). Forskolin, an adenylyl cyclase stimulator, showed positive inotropic (increase in contraction and calcium peak amplitude), chronotropic and lusitropic (decrease in relaxation time) effects. Negative inotropes showed decreases in calcium and contraction peak amplitudes. Verapamil also showed negative lusitropic and inotropic effects and altered electrophysiology. The calcium sensitizers/PDE3 inhibitors (levosimendan, pipomobendan) were classified incorrectly. The ability to simultaneously quantify multiple dynamic parameters in hiPSC-CMs provided crucial insights into electrophysiological and contractile drug responses. The assay was capable of detecting inotropic effects of compounds with various MoA. PDE3 inhibitors were not identified by the assay. This suggests that approaches to further advance hiPSC-CM culturing conditions or improving maturation are needed to further improve the predictive value of hiPSC-CMs in drug testing.

**1436 Assessment of Drug-Induced Liver Injury (DILI) Using 3D Human Liver Microtissues**

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Preclinical safety assessment is an essential step in drug development and drug-induced liver injury (DILI) constitutes a major hurdle for the progression of drug candidates to clinical studies. Thus, it’s critical to have predictive and scalable in vitro model systems for evaluating hepatic drug safety. Based on our proprietary technology, we developed human 3D liver microtissues (hLiMTs) as a highly predictive and scalable model system for drug safety assessment. Composed of primary human hepatocytes, and non-parenchymal cells (Kupffer and liver endothelial cells), hLiMTs display key physiological features and can be cultured up to four weeks for the assessment of long-term drug toxicity. A validation experiment analyzing 110 marketed drugs revealed that 3D hLiMTs exhibited a more than 2-fold increased sensitivity for detection of DILI compounds compared to 2D primary hepatocytes. Moreover, our system is compatible with the whole range of endpoints from classical biochemical assays, such as LDH leakage, albumin secretion, cell viability, omics (e.g., genomics, transcriptomics, lipidomics, etc.) and translational readouts such as AST. Thus, 3D hLiMT enable robust and predictive compound screening in a high throughput format to assess complex and mechanistic toxicity challenges. The 3D hLiMT model is available in 96-well and 384-well plates and may also be used in a plug-and-play organ-on-a-chip and a Microphysiological Platform System (Body on a Chip) to enable robust and predictive compound screening at a high throughput and scale as well as to assess complex, specific investigative toxicity challenges. In summary, we present that 3D hLiMT is a promising powerful alternative to 2D in vitro cell models and animal models that may be applied in preclinical safety evaluation of novel drug candidates, thus representing a valuable tool for pharmaceutical research.
A Novel 68-Gene Biomarker Signature to Identify Respiratory Irritation/Toxicity Induced by Inhaled Compounds Using Machine-Learning Assisted Classifications Based on Transcriptomic Data from Calu-3 Cells


An integrative discovery toxicology strategy, in which potential drug safety concerns are identified and mitigated early during drug discovery, is a key determinant of success for the development of novel innovative drugs. This requires the application of an arsenal of in vitro assays that predict clinical safety and provide mechanistic understanding of drug-induced toxicity. Novel therapeutically active agents for treatment of respiratory diseases, including asthma and chronic obstructive pulmonary disease, are often delivered by inhalation directly to the diseased tissue, which enables direct access to diseased tissue and low systemic exposure to minimize adverse effects in secondary organs. Therefore, there is a need to establish in vitro assays to screen for respiratory tract toxicity that can be employed during early stages of drug discovery. Since we have previously successfully established transcriptomic biomarkers for skin sensitization and respiratory sensitization, we explored the possibility to develop an in vitro assay for assessment of respiratory irritation/toxicity based on genomic biomarkers in chemically exposed cell cultures, with subsequent machine-learning assisted classifications. The lung-associated epithelial Calu-3 cells were exposed to a reference panel consisting of 18 compounds with a well characterized respiratory safety profile and whole genome expression data was obtained using microarray technology from biologically triplicated samples. Using a combination of analysis of variance and data-driven feature selection methods, a biomarker signature composed of 68 differentially regulated genes was established. The predictive functionality of the proposed biomarkers was verified using a leave-K-out Support Vector Machine (SVM) cross-validation exercise, establishing the predictive accuracy to 94%: 6 of 7 irritants and 11 of 11 non-irritants were correctly classified. In summary, we have established a novel 68-gene transcriptomic biomarker signature in a simple cell system, which can be employed to provide an early indication of respiratory irritancy/toxicity of inhaled drug candidates.

Controlled Conditions Reduce Critical Edge Effect in 96-Well Plates


The Edge Effect causes a substantial loss of usable assay space in pharmaceutical drug discovery assays conducted with 96-well culture plates. While mechanisms for reducing evaporation in edge wells have been employed to reduce variability in cell density, we have preliminary data suggesting that differences in temperature in edge wells while cells are settling may be a major contributing factor. Here we extend those results with careful temperature studies during and after cell plating processes with the premise that maintaining the temperature of all parts of the cell environment including plates, reservoirs, and tips, as well as the liquids at a constant 37 degrees C during cell plating will reduce edge effects. Our null hypothesis is that the Edge Effect cannot be reduced by comprehensive temperature control. We used the Xvivo System to control the environment, including the cell processing chamber floor, to a constant 37 degrees C during cell plating and cell settling, comparing results there to those obtained plating in a room temperature laminar flow hood. In both settings, we used a FLIR ONE thermal imaging camera to record movies of temperature changes in 96-well plates as well as the pipetting reservoir during and after routine cell plating. We used the HoloMonitor M4 microscope to record time lapse images of cells settling in the wells. We also used standard cell density assays to assess variability in plated adherent A549 human lung carcinoma cells. We found that plating cells under controlled, constant temperature conditions did eliminate swings in well temperatures during cell plating and cell settling that were produced by plating cells in uncontrolled room air conditions. Constant conditions also reduced variability in edge well cell density, disproving the null hypothesis. We concluded that constant conditions for cell plating and cell settling could reduce Edge Effect and, by allowing usage of all wells, have a tremendous impact upon the time and resources devoted to all cell-based toxicity assay processes.

A Novel 68-Gene Biomarker Signature to Identify Respiratory Irritation/Toxicity Induced by Inhaled Compounds Using Machine-Learning Assisted Classifications Based on Transcriptomic Data from Calu-3 Cells

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Background: Liposomal drugs have been recently approved that attenuate adverse effects and/or improve the efficacy of active pharmaceutical ingredients (APIs), such as Doxil (liposomal doxorubicin) and CPX-351 (liposomal formulation of cytarabine and daunorubicin). However, possible pathological responses due to the physicochemical properties of these liposomal formulations remain unknown. In the present study, we examined pathological changes in canine liver and gall bladder following injection of Doxil or empty liposome (a liposomal formulation without an API). Method: A single dose of Doxil or empty liposome was administered intravenously to eight-month-old beagle males (N=4/group), and gross necropsy was conducted at 24 hours post-injection. The liver and gall bladder were then examined histopathologically. Result and Discussion: Reddish foci were observed in the liver (sub-adventitia of gall bladder) and gall bladder (serosa from body to bottom) at necropsy in 2 of 4 males per treatment group. In histopathology, there were perivascular hemorrhage around the central/sublobular vein or Glisson’s sheath, dilated lymphatic vessels around the central vein in the liver, and hemorrhage in the adventitia of the gall bladder. Decreased cytoplasmic granules in mast cells beneath the endothelium of the hepatic vein were also noted. No differences were observed in the incidence and severity of the hemorrhagic changes between the two groups, suggesting that physicochemical properties of the liposomal formulations rather than the API caused these changes. These were considered as acute changes, because they occurred within 24 hours post-injection accompanying with little cellular infiltration. Based on these findings, we speculated physiological active substances released from mast cells contributed to the pathogenesis of hemorrhagic changes, and these changes may not occur in other experimental animals, which do not have subendothelial mast cells in the hepatic vein.

Toxicopathologic and Pathologic Evaluation of Cytochromes P450 Inhibition by 1-Aminobenzotriazole following Repeated Oral Administration in Wistar Rats


1-Aminobenzotriazole (1-ABT) is a pan-specific, mechanism-based inhibitor of cytochrome P450 enzymes, often used as co-treatment to investigate the metabolism-dependent toxicity of drugs and chemicals. To assess the confounding effects of 1-Aminobenzotriazole (ABT) in such kind of mechanistic studies, a repeated dose toxicity study with ABT following 7 days oral administration at 0, 25, 50 and 100 mg/kg/day was performed in Wistar rats (5 rats/sex/group). The parameters of standard general toxicity study viz. clinical signs, body weight, feed consumption, hematology, clinical chemistry, organ pathology, organ weight and histopathology was evaluated in this study. The 1-Aminobenzotriazole was tolerated up to the highest tested dose of 100 mg/kg/day. Neither clinical signs nor any mortality was observed at any dose tested. Slight increase in body weight gain was noted in ABT treated females. There was no significant change in food consumption throughout the study. Increased reticulocyte count and decreased triglycerides, BUN, A/G ratio and plasma potassium levels were noted in ABT treated animals. Increases in liver, kidneys, adrenals, and thyroid weights were noted in ABT treated animals. Microscopic findings included follicular cell hypertrophy in thyroid, hyper trophy in adrenal glands (zona fasciculata), basophilic cell hypertrophy in pituitary, and endometrial hyperplasia in uterus at all dose levels and hepato-cellular hypertrophy was noted at 100 mg/kg/day only. Based on this study it is concluded that 1-Aminobenzotriazole is tolerable up to 100 mg/kg/day with some changes in clinical pathology, organ weight and histopathology; these changes should be considered during the assessment of any mechanistic study with ABT.
Small Molecule Inhibitor of BRG1/SMARC4-ATPase (SWI/SNF Complex) in Phenotypic-Induced Gastrointestinal Toxicity with Possible Progenitor Cell Modulations in Athymic Mice


Members of the ATP-dependent SWI/SNF chromatin remodeling complexes are among the most frequently mutated genes in human tumors and their dysregulation plays critical role in carcinogenesis. Recent studies have shown a synthetic lethal relationship between BRM/SMARC2 and BRG1/SMARC4 in which BRG1-deficient cancer cells depend on BRM for proliferation. This discovery steered great interest in pursuing the therapeutic targeting of BRM for the treatment of BRG1-mutant/different cancers. We have discovered Compound-X, a dual small molecule inhibitor of BRM and BRG1 ATPase, as a candidate anti-cancer drug. Therapeutic potential of the Compound-X showed significant engagement through KRT80 messenger modulation and tumor growth inhibition in athymic mice. However, efficacy (decreased in implanted tumor xenograft sizes) was only observed at the dose associated with body weight loss, triggering study termination. Microscopic evaluation results revealed the poor condition of athymic mice from the study were due to the changes in the gastrointestinal (GI) tracts. In the intestines, there were altered villus architectures characterized by villus fusion/clumping with loss of discrete crypt structures. The intestinal epithelial cells were composed of relatively immature cells represented by increased cytoplasmic basophilia and large nuclei as well as decreased goblet cell populations. In the large intestine, there were ulcerations as well. Immunohistochemistry investigation results showed there was decreased Olfm4 (stem marker) expression in the ileum without expression change in proliferation index marker Ki67. Similar histopathological and Olfm4 expression changes are reported with intestinal tissue specific BRG1 Knock-Out mice. While we cannot completely rule out scaffold related off-target effects, these findings potentially inform concerns on off-target toxicity associated with dual BRM/BRG1 small molecule inhibition.

Cardiotoxicity Assessment of Triclosan in Human Stem Cell-Derived Cardiomyocytes

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Triclosan (TCS), as a broad-spectrum antibacterial agent, is frequently detected in the environment and humans. There is increasing evidence TCS can transport across the placenta and affect fetal heart development via maternal exposure. Herein, human embryonic stem cells (hESCs) based cardiac differentiation model was used to determine the cardiotoxicity of TCS. hESCs were subjected to a 21 days' differentiation protocol with TCS or control treatment. Cell morphology, spontaneous beating rates of cardiomyocytes (CMs), cardiac differentiation rates were recorded. Then gene transcriptome and genome-wide DNA methylation analysis were performed. TCS greatly inhibited CMs generation and the spontaneous beating rates of CMs. Differentially expressed genes (DEGs), including 917 up-regulated and 1246 down-regulated DEGs, were identified by RNA-seq. The GO analysis revealed that these DEGs were mainly involved in aberrant cardiac development pathways. DNA methylation status was also altered by TCS exposure. 424 hypo and 779 hyper differentially methylated regions (DMRs) were identified. Several heart development transcription factors and other cardiac marker binding sites were enriched in DMRs, including GATA family members. Further combining the DNA methylation and gene expression profiles, we found a group of down-regulated cardiac development genes exhibited hypermethylated sites. In conclusion, our findings suggest exposure to TCS stimulated a reprogramming of the DNA methylation in the hESCs-derived CMs, thereby orchestrating changes in gene expressions consistent with CMs development defects. In addition, hESCs-derived CMs model enables quantitative screening of the potential cardiotoxicity of environmental chemicals in a short-term experiment.

Possible Window of Susceptibility-Dependent Effects of Tretinoin on Human Cardiomyocyte Differentiation

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Retinoic acid is essential throughout a person’s lifetime, but it is critical during pregnancy. Tretinoin, also known as all-trans-retinoic acid (ATRA), is a naturally occurring derivative of vitamin A (retinol). It regulates cell proliferation and differentiation and can be used to treat acne and photodamaged skin. Tretinoin is also used in the treatment of acute promyelocytic leukemia (APL). However, during embryonic development, the effects of tretinoin at the cellular level could lead to teratogenic effects on the central nervous system and on heart development. Embryonic stem cells (ESCs) offer an excellent opportunity for studying the mechanisms of developmental toxicity. To explore the potential adverse effects of tretinoin on cardiomyocyte formation, we used an ESC line containing an Nkx2-5 reporter to model mesoderm differentiation and cardiac induction. Using an 8-day differentiation protocol, we examined the effects of tretinoin during different potential windows of susceptibility on cardiomyocyte differentiation. Tretinoin (EC50 = 0.001 μM; Cmax value 0.12 μM) suppressed expressed cardiac induction and cardiac differentiation. Across a range of concentrations (0.01 - 10 μM), cardiomyocyte differentiation was dramatically inhibited when the ESCs were exposed to tretinoin during the mesodermal induction stage (Days 0 to 2) of the differentiation protocol. However, when the ESCs were exposed only during the cardiomyocyte induction stage (Days 2 - 4 only), tretinoin had little to no effect on the differentiation of the ESCs to cardiomyocytes. Mesoderm-associated transcription factors, Musp1 and Mlx1, showed a transient up-regulation, while the transcription factors and mesoderm markers Hand1, Sna2, Hoxp, and cardiac-specific genes Nkx2-5, Gata4, Troponin T, and α-Actinin were all significantly suppressed during early tretinoin exposure (Days 0 - 2 only) compared with exposures during the later cardiomyocyte induction stage (Days 2 - 4 only). Together, these results suggest a specific window of susceptibility to tretinoin during cardiomyocyte differentiation, which could result in cardiac-related malformations initiated during pregnancy. Additional work is being conducted to further elucidate mechanisms involved in these aberrant effects, and to determine a possible NOEL of tretinoin on cardiomyocyte differentiation.

Comparative Analysis of Human iPSC-Derived Cardiomyocytes in Diversity and Disease Modeling

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Human cell types differentiated from induced pluripotent stem cells (hiPSCs) provide a unique access to human cellular material for safety and toxicity screening. Here, we present data demonstrating the utility of hiPSC-derived cardiomyocytes (hiPSC-CMs) in safety assessment and disease modeling. We include a comparative assessment of the human safety profile of CTRCD compounds doxorubicin (type I) and sunitinib (type II) across hiPSC-CMs derived from 6 healthy donors (DIV 14) at three concentrations [0.1, 1.0, and 10 μM]. Clinically type I CTRCD may be associated with cellular death, structural changes, and permanent damage while type II CTRCD may be associated with cellular dysfunction, no structural changes, and reversible damage. Here, we identify both type I and type II CTRCD using a selected in vitro cohort of hiPSC-CMs. These data further provide additional insight into sensitivities to cancer therapeutics across different donors. In addition, we elucidate basic functional characterization and pharmacological response for several hiPSC-CM disease models including hypertrophic cardiomyopathy MYH7 (R403Q), LMNA-related dilated cardiomyopathy LMNA (L35P), and brugada syndrome type 3 CACNA1C (G490R) each with its respective isogenic control at DIV 14. We further identify the functional consequences of each mutation and demonstrate that each model recapitulates classical hallmarks of the disease. These data illustrate how hiPSC-CMs provide an excellent model system for assessing compound effects across multiple donors and disease models. Taken together, these examples should help to create new avenues for safety liability assessment and toxicology studies, as well as serve as a template for future opportunities in disease modeling with hiPSC-CMs.

Mechanisms of Cadmium and Arsenic-Induced Aberrant Differentiation of Human Embryonic Stem Cells to Cardiomyocytes during Heart Development

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Cadmium (Cd) and arsenic (As) are widespread environmental contaminants. Human exposure to Cd and As occurs mainly through ingestion of contaminated food or water. Cd and As exposure are associated with cardiovascular diseases, and maternal exposure to Cd and As are significant risk factors for congenital heart disease (CHD). However, the mechanisms of Cd and As on developmental cardiotoxicity are not well-defined. Embryonic stem cells (ESCs) offer an excellent opportunity for studying the mechanisms of developmental toxicity. 2D aggregates of ESCs, called embryoid bodies (EBs), can recapitulate events involved in early embryogenesis such as germ layer formation. Here, we found that a 7-day exposure to a human-relevant, non-cyto-
toxic dose (0.6 μM; 100 ppb) of Cd inhibited differentiation of EBs to ectoderm and mesoderm via suppression of Wnt/β-catenin signaling pathways, which play critical roles in early embryonic development. We also found As suppressed key regulators involved in Wnt/β-catenin signaling pathways at a non-cytotoxic dose (0.5 μM; 35 ppb). Human atrial and ventricular cardiomyocytes derive from mesoderm populations. NTRK-2-5 is a transcription factor that plays key roles throughout heart development and formation, and mutations can lead to atrial and ventricular defects. To explore potential adverse effects of Cd and As on cardiomyocyte formation we used a 2D ESC line containing an NTRK-2-5 reporter to model mesoderm differentiation and cardiac induction. Using an 8-day differentiation protocol, Cd (0.15 μM; 27 ppb) and As (0.05 μM; 6.5 ppb) suppressed cardiomyocyte differentiation. Mesoderm-associated transcription factors MESP1, MIXL1, EOMES, and GSC showed a transient upregulation, while the transcription factors and mesoderm markers HAND1, SNAI2, HOPX, and cardiac-specific genes NTRK-2-5, GATA4, Troponin T, and α-Actinin were all suppressed when treated with Cd. In conclusion, low concentration Cd suppressed mesoderm formation through Wnt/β-catenin signaling pathways and thus inhibited downstream cardiomyocyte differentiation and cardiac induction. As inhibited cardiomyocyte differentiation specifically through key genes in Wnt/β-catenin signaling pathways. These studies provide valuable insights into the cellular events and molecular mechanisms associated with Cd and As-induced CHD.

**1446 Gene-Environment Interaction of DDT/ DDE and APOE on the Amyloid Pathway in Induced Human Neurons**

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The E4 allele of the Apolipoprotein E (APOE) gene contributes the greatest single genetic risk for late-onset Alzheimer Disease (LOAD), but it is also affected by factors including age and environmental exposure. The pesticide dichlorodiphenyltrichloroethane (DDT) has been identified as a risk factor for LOAD due to its persistent bioaccumulation and elevated serum levels of the DDE metabolite, dichlorodiphenyltrichloroethylene (DDE) of AD patients, even after it was banned in the U.S. over forty years ago. Further, we observed that there were significant interactions between serum DDE levels, APOE genotype and cognitive dysfunction, with APOE ε4 genotype and higher DDE levels being associated with worsened cognitive function. Mechanistically, our previous experiments found significant increases in amyloid precursor protein (APP) levels in differentiated SH-SY5Y cells and primary mouse hippocampal neurons after DDT/DDE exposure. To confirm this observation in a human neuron model, we prepared induced neurons (iNs) by reprogramming induced pluripotent stem cells (iPSC) with NGN2. To test the hypothesized interaction of DDT/DDE exposure with APOE genotype, we used two isogenic iPSC lines, one with APOE ε3/ε4 and one engineered to carry inactivating frameshift mutations in both alleles (APOE-null). Preliminary results show that 24 hr treatment of heterozygous ε3/ε4 iNs with 1 μM DDT or DDE exhibited increased expression of APP in neurons, as measured immunocytochemically. Furthermore, we observed a synergistic interaction of DDE and APOE genotype, since APP expression was uniformly reduced in the APOE-null neurons and there was no evidence of an induction of APP by DDT or DDE. Results suggest that DDT/DDE acts in conjunction with APOE, altering the expression of APP, potentially increasing neurotoxic Aβ and contributing to the development of AD.

**1447 High-Throughput Toxicity Screening of iPSC-Derived Neurons on Synthetic Hydrogels**

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The developing central nervous system is often more susceptible to damage than the adult one, especially through exposure to neurotoxicants. Of the over 80,000 chemicals currently available, only 200 have undergone developmental neurotoxicity (DNT) testing according to established guidelines. The use of animal models in predicting neurotoxicity (NT) is costly, time consuming, and results often fail to translate to human physiology. Other culture scaffolds such as Matrigel introduce concerns regarding variability between batches, providing the need to develop a synthetic substrate for in vitro modelling of NT using human cell models. In this study, we evaluated neurite outgrowth as a potential screen to determine the effects of compounds on the developing nervous system. To model neurogenesis in vitro, we cultured human iPSC-derived neurons on poly(ethylene glycol) hydrogels functionali zed with CRGDS and IKVAV peptide sequences in a poly-L-lysine coated 384-well plate. After culturing the neurons for 3 days on hydrogels, we treated them with neurotoxicants at 0.1, 0.4, 1.6, 6.25, 25, and 100 μM concentrations. Multiple DMSO controls were included in each plate. Neuronal retraction due to exposure to compounds was studied over the course of 5 days. Networks were characterized and validated with neuron-specific cytoskeletal protein (βIII Tubulin, MAP-2) markers. Our results showed significant neurite regression in response to the drug thalidomide at high concentrations. Additionally, neuronal network analysis found a decreasing trend in total network area as concentrations increased. Our high-throughput screening avoids the concerns surrounding the use of animal-derived substrates while capturing the physiologically-relevant process of neuronal network formation in the development of the nervous system.

**1448 Classification of Drug-Induced Seizure-Like Activities in Cultured Human iPSC-Derived Neurons**


Human induced pluripotent stem cell-derived neurons are promising for use in toxicity evaluations in nonclinical studies. One of the major adverse events affecting the central nervous system observed during clinical trials is convulsions. Micro-electrode array (MEA) systems have recently attracted attention for use in evaluating the convulsion potential of a drug because they non-invasively measure the electrophysiological activity of neural networks at multiple sites in a high-throughput manner. MEA subteam of Neurotoxicity (NeuTox) Committee in Health and Environmental Science Institute (HESI) initiated the NeuTox Micro-Electrode Array (MEA) Subteam initiated pilot study using MEA for the prediction of seizure liability of drugs. Human iPSC-derived cortical neurons were cultured on 24-wells MEA plate for extracellular recording using Presto. Twelve compounds (pentylentetrazole, picrotoxin, 4-aminoypyridine, linopirdine, amoxapine, styrchicine, plicarpine, amoxicillin, chlorpromazine, enoxacin, phenytoin and acetaminophen) were tested at 5 concentrations for each compound. Using spontaneous firings data to drug administration, we identified the parameter sets that can separate the responses between convulsive drugs and negative control, and the responses among the several convulsiung drugs, differentiating with different principal component analysis and clustering methods. These analysis method will be effective for detecting convulsive response and predicting mechanism of action of convulsive drugs.

**1449 TRP Channel Responses in Human iPSC-Derived Sensory Neurons Using MEA System**


Functional evaluation assays using human induced pluripotent stem cell (hiPSC)-derived sensory neurons are expected to predict the pain-related toxicity of drugs and the pharmacological effects. However, evaluation assays in hiPSC-derived sensory neurons has not been established, and transient receptor potential (TRP) channel responses to pain-related molecules are also not known. In this study, we aimed to evaluate the TRP channel responses against pain-related molecules including anticancer drugs in cultured hiPSC-derived sensory neurons using high-throughput multi-electrode array (MEA) system. Human iPSC-derived sensory neurons were cultured on MEA chips (Presto), and the electrophysiological responses against capsaicin, menthol, allyl isothiocyanate (AITC), anticancer drug vincristine and oxaliplatin were measured by the MEA system. We firstly confirmed the expression of typical sensory neuron marker Nav1.7, TRPV1, TRPM8, and TRPA1 using immunostaining in culture hiPSC-derived sensory neurons at 8 weeks culture. Evoked responses against capsaicin, menthol, and AITC administration were detected using MEA system. To confirm the responses depending on each TRP channel, we examined the responses in presence of each channel antagonist. As the responses almost disappeared in presence of each channel blocker, these responses were confirmed to be TRP channel specific responses. The evoked responses against anticancer drug vincristine and oxaliplatin administration were also detected. Next, we examined whether the increase of cold sensitivities occur in presence of anticancer drug oxaliplatin in vitro hiPSC-derived sensory neurons. The responses against AITC were increased in presence oxaliplatin and in a concentration-dependent manner. In summary, we have succeeded in detecting the electrophysiological pain responses against capsaicin, menthol, allyl isothiocyanate (AITC), anticancer drug vincristine and oxaliplatin in hiPSC-derived sensory neurons using MEA system. We found that the increase of cold sensitivities in vivo phenomenon was also detected in vitro hiPSC-derived sensory neurons. MEA measurements using hiPSC-derived sensory neurons are useful to pain evaluation assay in human peripheral nervous system.
Cigarette smoking is associated with several types of cancers including hematological malignancies and a relationship between smoking and the risk for myeloproliferative neoplasms (MPN) has been shown. The mechanisms of smoke induced impairment of the hematopoietic system is thought to occur via the chronic inflammatory and pro-oxidant state linked to cigarette use. We hypothesize that cigarette smoke induces a pro-oxidant and pro-inflammatory state in the bone marrow leading functional changes in hematopoietic cells. Aqueous cigarette smoke extract (CSE) was prepared by drawing the mainstream smoke from 1 cigarette by continuous negative pressure into 10ml of buffer to produce 100% CSE. Murine bone marrow cells (BMcs) and human peripheral blood mononuclear cells (PBMCs) were isolated using standard methods and treated with CSE for 24 hrs with or without N acetylcytysteine (NAC). Colony formation in methylcellulose and cell viability by Annexin V and propidium iodide staining were determined in exposed cells. Reactive oxygen species (ROS) was determined using theDCF fluorescent dye on cells exposed for one hour. BMcs treated with CSE showed a dose dependent increase in ROS which was reduced on co-incubation with NAC. Cell viability was also decreased in CSE treated cells compared to controls. There was a profound decrease in the number of progenitor cell colonies formed by bone marrow cells exposed to CSE which was rescued by co-incubation with 100μM NAC. PBMCs from both normal and MPN subjects also exhibited reduced colony formation in response to CSE which was inhibited by incubation with NAC. We observed that CSE has an in vitro inhibitory effect on hematopoiesis accompanied by an increased redox status and cytotoxicity. Future studies will examine the cytotoxic responses and signaling cascade of BMcs and PBMCs in response to smoke extract and the effects of E-cigarette smoke condensate.

In order to investigate the mechanism of busulfan-induced chronic hematotoxicity, busulfan was intraperitoneally administered to female C57Bl6/JNcSic (CD45.2) mice (3 times/week, total 10 times, 10 ml/kg) at 0.5, 4.5, or 8 mg/kg. Hematological examination was conducted 1, 2, 3, and 4 months after the completion of dosing and bone marrow analysis by flow cytometry was performed 1 and 4 months after dosing. Moreover, a competitive bone marrow transplantation was conducted to evaluate the stem-cell and multipotent differentiation of hematopoietic stem cells (HSC) using C57BL/6 mice congenic for the Ly5 locus (CD45.1 mice) and lineage negative, c-kit positive, and Sca-1 positive cells (LSK) collected from CD45.2 mice 4 months after dosing. Furthermore, gene expression analysis was conducted in HSC and LSK at 4 months post-dose. In hematology, continuous decreases in red blood cells (RBC) and platelets (PLT) were observed at 4.5 and 8 mg/kg during the study period. In flow cytometric analysis of the bone marrow, decreases in HSC, multipotent progenitors (MPP) 3 and 4, megakaryocyte progenitors (Pre-MegE and MKP), and erythroid progenitors were observed at 4.5 or 8 mg/kg even at 4 months post-dose. Cell cycle analysis showed that quiescent cells in CD34 negative LSK decreased at 4.5 and 8 mg/kg 4 months after dosing. In bone marrow transplantation, chimerism of myeloid cells, B cells, and T cells in peripheral blood were lower than those in the corresponding control from 1 to 4 months after the transplantation. Moreover, chimerism of HSC, granulocyte-macrophage progenitors, megakaryocyte-erythroid progenitors, and common lymphoid progenitors in the bone marrow also decreased even at 4 months post-transplantation. Gene expression analysis revealed that cell cycle was activated and stem cell function and differentiation potential into erythroid lineage and megakaryocytes were inhibited in HSC or LSK. In conclusion, busulfan induced impaired self-renewal and multipotency of HSC, followed by decreased megakaryocyte progenitors and erythroid progenitors resulting in continuous decreases in RBC and PLT in mice.
Cell heterogeneity is a feature of cancer cell populations. Total cell population behaviour in response to a particular environment may not be replicated in a specific group of cells or sub-population. Determining which sub-population, if any, displays a specific biological response, and whether the plasticity of a group of cells within the population could be influenced by the environment is important for understanding biological response. To investigate this, the gastric cancer cell line SNU1, was treated with benzo[a]pyrene (BaP), a potent IARC Group 1 human carcinogen, to induce phenotypic response including DNA damage, confirmed by micronucleus assay. In addition to established BaP-AhR-associated genes (CYPs) (i.e. CYP1a1, CYP1b1, AhR, AHRR, ARNT), key genes commonly associated with epithelial to mesenchymal transition (i.e. SNAIL-1) and cell stemness (i.e. Oct3/4, Nanog and Sox2) were also temporally induced by BaP (10^{-7} M to 10^{-5} M). We suspected that certain sub-populations within the SNU1 total population were influencing biological function. Therefore, flow cytometry was used to observe the expression of cell surface marker of two BaP-treated sub-populations, CD24 and CD166. Each sorted population of CD24+CD166+ and CD24+CD166− shows different regulation of genes associated with stemness and BaP-induced phenotypic change. CNTNB1, a target gene of Wnt/β-catenin, was upregulated in the unsorted SNU-1 population, down-regulated in the sorted population of CD24+CD166+ population (BaP 10^{-7} M to 10^{-6} M) and remained unchanged in the sorted population of CD24+CD166−. Moreover, the induced expression of AhR-related and stemness genes found in the sorted CD24+ population, potentially indicates that this is the most probable candidate contributing to the plasticity of SNU-1. The co-existence and interaction of sub-populations within the SNU-1 cell culture plays a significant role in cell plasticity and in sustaining key signalling pathway responses and maintaining relative stemness of SNU-1 when it is exposed to BaP. Thus, our data suggest that in order to understand cell population physiology in response to perturbation of the environment, it is necessary to consider cell heterogeneity and that the relative plasticity of gastric cancer cells could be a contributing factor in BaP-induced gastric carcinogenicity.

Mercury (Hg) is considered by the World Health Organization (WHO) as one of the top priority chemicals that affect human and ecologic health worldwide. Although WHO recommends a constant concentration ratio (250:1) of hair-to-blood Hg for assessing mercury exposure, the mechanisms underlying variability in that ratio remain to be elucidated. The objective: to identify key determinants of the hair-to-blood ratio of total mercury in US women. The data were obtained from 1306 women aged 18-49 years, each of whom provided valid measurements of blood and hair Hg, in the National Health and Nutrition Examination Survey 1999–2000. The impact on the hair-to-blood ratio of factors, such as age, race, body mass index, socioeconomic status, seafood consumption, liver function (assessed by serum levels of alanine aminotransferase and aspartate aminotransferase), hair treatment, and smoking, were examined by linear regression analysis. Logarithmic transformations were applied when necessary. Although hair Hg level is well correlated with blood Hg concentration (Spearman correlation = 0.71, p < 0.05), there is a great deal of variability in the hair-to-blood Hg ratio in the study population (mean = 282; median [interquartile range] = 215 [129-330]). Of the physiological and sociodemographic parameters investigated, race is the leading factor followed by smoking in determining the hair-blood Hg ratio that while African-American women had the lowest estimated ratio, women in “All Other Races (including Asians)” have the highest hair-blood Hg ratio. Moreover, the hair-blood Hg ratio is approximately 13% higher among current and former smokers than their counterparts after covariate adjustment. On the other hand, the hair-blood Hg ratio among subjects with impaired liver function is 16% lower (p=0.08) than in subjects with normal liver enzymes. While this study generally supports the WHO recommendation of using a hair-to-blood Hg ratio of 250 for Hg assessment, one may need to consider contributing factors to the variation in the ratio. Further research (e.g., toxicokinetic modeling) would be informative to confirm our findings. A better understanding of the key determinants in the relation between levels of Hg in hair and blood could further improve mercury exposure assessment.
Intraperitoneal arsenic (iAs), a common drinking water and food contaminant, is associated with a variety of diseases including cancers, cardiovascular disease, and metabolic disorders. Although the mechanisms underlying iAs-associated illness remain poorly characterized, a growing body of literature raises the possibility that microRNAs (miRNAs), post-transcriptional gene suppressors, may serve as mediators and/or early biomarkers of the adverse effects of iAs exposure. To characterize the circulating miRNA profiles of individuals chronically exposed to iAs, samples of plasma were collected from 109 healthy residents of the Zimapán and Laguna regions in Mexico who were exposed to iAs in drinking water. These plasma samples were analyzed for small RNAs using high-throughput sequencing, and for iAs and its monomethylated (MAs), and dimethylated (DMAs) metabolites. Associations between plasma levels of arsenic species and miRNAs were evaluated. Six circulating miRNAs (miR-423-5p, -142-5p, -423-5p +1, -320c-1, -320c-2, and -454-5p), two of which have been previously reported as candidate biomarkers of cardiovascular disease and diabetes (miRs-423-5p, -454-5p), were found to be significantly associated with plasma MAs. No miRNAs were associated with plasma iAs or DMAs after correction for multiple testing. These miRNAs may represent mechanistic links between iAs exposure and disease or serve as biomarkers of disease risks associated with this exposure.

Circulating miRNAs as Potential Biomarkers of Arsenic Exposure

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Development of Circulating Exosome-Based Biomarkers for Manganese Neurotoxicity in Human Serum and Plasma Using RT-QuIC Assay and Exosomal RNA-sequencing Analysis

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Chronic exposure to Mn is known to cause neurological, Parkinsonian-like symptoms in exposed individuals by affecting the extrapyramidal motor control system. Occupationally exposed individuals like welders are at high risk due to constant exposure to Mn-rich welding fumes. Recently, we demonstrated that Mn interacts with a-synuclein and promotes its aggregation in cell culture and animal models of Mn neurotoxicity. No blood-based biomarkers are available for Mn poisoning in humans except for expensive imaging techniques, like brain MRI. In the present study, we developed a rapid, sensitive quantification method that detects a-synuclein aggregation by a real-time quaking-induced conversion (RT-QuIC) assay in Mn-exposed individuals. First, we generated a high-quality recombinant human wild-type a-synuclein protein as a substrate for this assay. Next, we optimized RT-QuIC assay conditions to further quantify a-synuclein-seeded aggregation in vitro models of a-synucleinopathies. Moreover, we determined the diagnostic utility of the a-synuclein RT-QuIC assay using exosomes isolated from a blinded cohort of serum and plasma samples from a population exposed to welding fumes and age-matched controls. We could differentiate welders from controls with >95% sensitivity and specificity with the RT-QuIC assay, suggesting that exosomal a-synuclein aggregates may serve as a circulating biomarker for Mn neurotoxicity. Furthermore, next-generation sequencing of serum exosomes showed changes in several small RNAs, such as miRNAs, piRNAs, and trRNAs that may potentially contribute to Mn neurotoxicity and thus could also evolve as potential biomarkers. Collectively, our findings demonstrate the use of a sensitive, high-throughput RT-QuIC assay for discovering a-synuclein-based biomarkers and identify potential exosome-associated small RNA biomarkers. Acknowledgements: ES 026892, Lloyd and Armbrust endowed.

The Noonan Syndrome - A Further Update


Electronic cigarette use has increased exponentially in the past decade, especially among young adults, with little knowledge about its long-term health effects. The objective of this study was to determine if urinary biomarkers of harm are elevated in electronic cigarette users and if elevation correlates with increased concentrations of metals. This was a cross-sectional study of biomarkers of harm in urine from 53 human participants separated as non-smokers (n=20), electronic cigarette users (n=20), or cigarette smokers (n=13). Ages ranged from 19-75 years with an equal number of males and females. All participants in each group were gender and age matched. Urine samples were collected from a previous study. The inclusion criteria for electronic cigarette users or cigarette smokers required exclusive and habitual use of either electronic cigarettes or conventional cigarettes for a minimum of 6 months, abstinence from smoking any other substance for at least 6 months, and no known health issues. Biomarkers of oxidative harm (8-isoprostane, 8-OHdG) and metal exposure (metallothionein), as well as metal concentrations were measured in the urine of non-smokers, conventional cigarette smokers, and electronic cigarette users. The lipid peroxidation biomarker, 8-isoprostane, showed a significant increase in electronic cigarette users (750.8 ± 433 pg/mg) vs non-smokers (411.2 ± 287.4 pg/mg). The biomarker of DNA oxidation, 8-OHdG, was significantly elevated in electronic cigarette users (442.8 ± 300.7 ng/mg) vs non-smokers (221.6 ± 157.8 ng/mg). Metallothionein, a biomarker of metal and reactive oxygen species (ROS) exposure, was significantly elevated in the electronic cigarette group (3761 ± 3932 pg/mg) compared to the non-smokers (1129 ± 1294 pg/mg). All three biomarkers in the electronic cigarette users were similar and not lower than cigarette smokers. Linear correlation plots showed correlations between cotinine and total metals, metallothionein and total metals, and total metals and oxidative DNA damage in the electronic cigarette users. The results of this study are the first to show a significant elevation in biomarkers of harm correlated with elevated metals in the urine of electronic cigarette users.

A Whole Blood Gene Expression-Based Diagnostic Test Prototype for Determining Smoking Status

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Smoking is a major risk factor for the development of diseases. A new generation of smoke-free products for adult smokers have been designed to significantly reduce the formation of toxicants in the aerosol, potentially resulting in reduced exposure to toxicants. To assess whether this reduced exposure translates into beneficial molecular changes, it is essential to identify reliable molecular biomarkers, easily measurable in human subjects, that can discriminate between smokers (S) and non-smokers. The DxDirect technology platform (DxTerity Diagnostics Inc.) enables measurement of 25 genes from isolated RNA or a stabilized fingerstick blood sample. The DxDirect chemical ligation reaction generates uniquely sized ligation products corresponding to each mRNA, which, following polymerase chain reaction amplification, are quantified using a Thermo Fisher ABI 3500Dx CE instrument. For each mRNA target, two chemically reactive exon-exon junction-spanning probes were manufactured and tested. A linear discriminant analysis predictor algorithm was developed from known isolated RNA training samples, and the final prototype multiplex test was evaluated blindly or not using RNA and blood samples from independent cohort studies. Using various human whole blood transcriptomic data and computational approaches, we identified a robust core signature, including genes such as AHR, CDKN1C, LRRN3, PID1, GPR15, and SASH1, that differentiates S from non-smokers. Using DxDirect technology, a subset of 25 genes were multiplexed in an assay to develop a gene expression-based diagnostic test prototype that discriminates S from non-smokers. Initial testing of the prototype shows a significant separation between S and non-smokers (NS) with both RNA and blood sample types. RNA samples from former smokers (FS) were classified between S and FS, however, with scores closer to those computed for NS. In conclusion, we have developed a gene expression-based diagnostic test prototype that demonstrated the capacity to determine smoking status in human subjects from RNA and blood drop. Further evaluations will determine the impact of potential confounders on the performance of the diagnostic test.
**1462** Relationship between Neurobehavioral Changes and Drug Concentrations in CSF in Cynomolgus Monkeys


Cerebrospinal fluid (CSF) has been used for research of biomarkers and development of new drugs for the central nervous system (CNS); however, there are few reports on the relationship between behavioral changes and drug concentrations in CSF. We have developed a new method for serial sampling of CSF from conscious cynomolgus monkeys. The purpose of this study is to evaluate the relationship between neurobehavioral changes and drug concentrations in CSF from cynomolgus monkeys. Nine male cynomolgus monkeys with a catheter for CSF sampling previously implanted into the cisterna magna through the spinal subarachnoid space were divided into 3 groups: control (0.5% methylcellulose aqueous solution) and diazepam 6 and 30 mg/kg (n=3, orally). Functional observational battery (FOB) observation and sampling of both plasma and CSF were conducted pre-dose and at 0.5 (sampling only), 1, 2, 4, and 24 h post-dose. Diazepam concentrations in CSF and plasma were determined using LC-MS/MS. Results Animals administered diazepam at 6 mg/kg showed decreased locomotor activity, ataxic gate, flaccidity, hypothermia, and sedation 1 h post-dose and these changes were continued until 8 h post-dose. The plasma level reached the peak 1 h post-dose and decreased thereafter (plasma range: 8.55 to 390.8 ng/mL). The level in CSF showed similar changes but lower than in plasma (CSF range: 0.64 to 15.43 ng/mL). At 30 mg/kg, neurobehavioral changes and drug concentrations in plasma and CSF were almost similar to those at 6 mg/kg; however, 1 animal showed somnolence at 2, 4, and 6 h post-dose, and drug concentrations in CSF at these time points were higher (2 h: 11.32 ng/mL; 4 h: 36.98 ng/mL; 8 h: 25.89 ng/mL) than those in other animals. At 24 h post-dose, abnormal behavior including flaccidity nearly disappeared at 6 mg/kg, and drug concentrations in plasma and CSF were low (plasma: 1.13 to 13.43 ng/mL; CSF: 0.13 to 0.88 ng/mL). At 30 mg/kg, decreased locomotor activity, flaccidity, and ataxic gate remained in all animals, and drug concentrations were higher than those at 6 mg/kg (plasma: 55.95 to 309.6 ng/mL; CSF: 2.52 to 9.87 ng/mL). Drug concentrations in CSF could reflect neurobehavioral changes induced by diazepam, and sequential sampling of CSF from cynomolgus monkeys could be useful for CNS research.

**1463** Proteomic and Metabolomic Profiling Identify Plasma Biomarkers for Exposure to Ultra-Low Levels of Carfentanil

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Despite the epidemic of opioid abuse, there are few validated assays capable of rapidly detecting these compounds. Carfentanil, in particular, is nearly undetectable at physiologically relevant concentrations. Therefore, we developed a systems biology approach to discover host-based markers that were specifically upregulated upon exposure in a rabbit model. Rabbits were exposed to 0.18 mg/ml of carfentanil via nose-only chamber. Blood was taken from each animal (N=6) pre-exposure, immediately post-exposure, +6 hrs, 1 day (D), 2D, 3D, 6D, 7D, 8D, 9D, and 13D. The blood was spun down to separate the plasma and CSF. Untargeted proteomics and metabolomics analysis of both plasma and CSF were conducted pre-dose and at 0.5 (sampling only), 1, 2, 4, and 24 h post-dose. The sites of glycosylation were determined as asparagine-35 (minor),134 and asparagine-135 residues were evaluated. The N-glycosylated asparagine sites of which two consensus (asparagine-37 and 134) and one non-consensus (asparagine-135) residues were evaluated. The N-glycosylated asparagine residues were converted to aspartic acid by the action of the PNGase F, which resulted in an increase in molecular mass of 0.984 Da per glycosylation site. The sites of glycosylation were determined as aspirgine-35 (minor),134 and 135 by use of trypsin digestion coupled with analysis of the resulting peptides by nano-UHPLC/HRMS. The approach is now being applied to the analysis of plasma HMGB1 from control healthy subjects and mesothelioma patients. Supported by P42ES027720 and P30ES013508.

**1464** Benzop(a)pyrene (BaP) Increases LINE-1 mRNA Export in Lung Epithelial Cell Exosomes


Although the link between cigarette smoking and lung cancer has been well-established, the causal mechanisms are still being defined and novel biomarkers are needed to improve lung cancer screening. We hypothesize that Long Interspersed Nuclear Element 1 (LINE-1) levels in lung epithelial cell exosomes may serve both as a mechanisms-based biomarker of smoking-related carcinogenesis. Exosomes are secreted membrane-bound vesicles that can regulate gene expression in recipient cells via paracrine mechanisms. LINE-1 is a DNA retroelement that can ‘copy and paste’ itself and other DNAs into different loci via a reverse transcriptase-mediated mechanism. This genomic element is epigenetically silenced in healthy cells and activated by harmful environmental exposures, such as tobacco smoke. LINE-1 activation initiates oncogenic phenotypes and is also a hallmark feature of cancer cells. In studies reported here, we challenged lung epithelial cells for 48 hours with different concentrations of benzo(a)pyrene (BaP), a cigarette smoke carcinogen that induces LINE-1. Both cells and exosomes were harvested and LINE-1 DNA and mRNA levels were quantified by qPCR. BaP increased cellular LINE-1 levels 2.5 fold with induction profiles that were mirrored in exosomes. LINE-1 mRNA levels in each cell released between 100-400 copies of LINE-1 DNA into exosomes, but no significant BaP-related trends were observed. Together, these results support the hypothesis that the LINE-1 status in lung epithelium can be inferred by examining lung epithelial cell exosomes, and suggest the eventual use of exosomal LINE-1 content as a biomarker of environmentally-related disease. Furthermore, the exosome-mediated transport of LINE-1 mRNA into neighboring cells may provide a vehicle for exposed cells to induce oncogenic phenotypes in healthy cells.
1466 BaP Exposure Increases LINE-1 ORF1 Protein Export in Lung Epithelial Cell Exosomes
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Exosomes are vesicles of endocytic origin that can induce physiological changes in recipient cells. At such, exosomes may have an undefined role in mediating the effects of chemical exposures in tissues that significantly influence health outcomes. There is compelling evidence connecting cigarette smoking and lung cancer; however, the mechanisms and novel biomarkers are still being defined. We hypothesize that Long Interspersed Nuclear Element 1 (LINE-1) proteins inside lung epithelial cell exosomes serve as a biomarker and mediator of smoking-induced lung cancer. We report here findings that can replicate and insert itself into different loci through reverse transcription. LINE-1 is epigenetically silenced in healthy cells and activated by harmful environmental exposures such as tobacco smoke. Furthermore, LINE-1 activation stimulates oncogenic phenotypes in lung epithelial cells. To investigate this hypothesis, we treated lung epithelial cells with different concentrations of benzo(a)pyrene (BaP), a cigarette smoke carcinogen known to induce LINE-1, and quantified levels of LINE-1 protein (ORF1) in cells and exosomes by Western blotting. We found that BaP increased ORF1 levels up to 2.3-fold in treated cells compared to vehicle controls. We discovered that ORF1 protein was secreted into extracellular media. These findings support the hypothesis that LINE-1 protein levels in exosomes serve as a biomarker of environmental exposures associated with lung cancer.

1467 Nephrotoxic Biomarkers in Gentamicin-Induced Acute Kidney Injury for Hazard Identification
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Bioliquid-based microRNAs (miRNAs) have been explored as biomarkers for predicting toxicity of chemicals. Here, we evaluated that miRNA biomarkers could be employed as nephrotoxic endpoints in vivo acute kidney injury models. For this, the SD rats were subcutaneously administered daily with gentamicin sulfate (single dose of 400 or 600 mg/kg, and repeated doses of 200 or 600 mg/kg per day) for 7 days. Acute kidney injury was confirmed by typical endpoints (the elevation of blood urea nitrogen, serum creatinine and urinary kidney injury molecule-1, or the presence of histopathological lesion). About 50 genes were selected as literature-based candidate miRNA biomarkers by reviewing previous studies. Among these genes, miR-378a-3p was significantly upregulated in serum and urine of rats administered to repeated doses of gentamicin sulfate (600mg/kg). In addition, miR-26b-5p, miR-34a-5p, miR-320-5p and miR-345-3p were increased with more than 3-fold changes in rat urine of same treatment groups. These findings suggested that miRNA biomarkers may be useful as additional endpoints for predicting nephrotoxic potential of chemicals.

1468 Assessment of Welding Fume Exposure on Telomere Length and Regulation in Peripheral Blood Mononuclear Cells and Lung Tissue in Rats

Telomeres are DNA fragments at the ends of chromosomes that protect genetic information during cell proliferation. Telomeres control cell DNA damage response (DDR) and DNA repair activity during cell division by regulating ATM and ATR kinases. Protection of telomeres 1 (POT1) protein specifically binds the 3’ overhang of the telomere and plays a key role in chromosomal end protection and telomere length regulation. In this study, we examined POT1 mRNA expression and telomere length and regulation by shelterin complex proteins in peripheral blood mononuclear cells (PBMCs) and non-lung whole lung tissue in male Sprague-Dawley rats following exposure, by intratracheal instillation, to 2 mg/rat of manual metal arc-stainless steel welding fume (WF) particulate or saline (vehicle control). PBMCs and lung tissue were harvested at 30 days after exposure. For 48 hours with different metals (41% Fe, 28% Cr, 17% Mn, 3% Ni) with a mean count diameter of 600 nm. The PBMCs recovered from WF-exposed animals had increased telomere length as analyzed by fluorescent in situ hybridization (FISH), flow cytometry, and qPCR compared to controls. Altered expression of shelterin regulatory proteins, tripeptide-peptidase 1 (TPP1) and TERF1-interacting nuclear factor 2 (TIN2), was observed in PBMCs and lung tissues. Additionally, increased telomere length in lung tissue as analyzed by qPCR was observed in the WF group compared to control. However, qPCR analysis showed that POT1 expression levels in the lung tissue of the WF group relative to those in the control group (T/N ratio) was significantly lower, leading to the activation of ATR expression that was not observed in PBMCs. These results indicate that exposure to WF down-regulated lung POT1 which in turn activated ATR-dependent DNA damage signaling and telomere elongation in the lungs, as well as activation of telomerase-independent pathway, an alternative mechanism leading to telomere elongation in PBMCs.

1469 miRNA Profile Assessment of Urine Exosomes from Boric Acid Treated Rats as Potential Biomarkers for Testicular Toxicity
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The objective of this study is to identify miRNAs in urine specific to boric acid (BA) related reproductive effects in rats. Urine samples were evaluated to identify differentially expressed testis-specific miRNAs in BA treated rats using next-generation sequencing (NGS). Boric acid was administered to male Sprague Dawley rats via oral gavage at a dose level of 500 mg BA/kg bw/day for 28 days, a dose level known to produce fertility effects in male rats with minimal overt signs of toxicity. At the end of 28-days urine was collected over 24 hours. Urine samples were shipped to QIAGEN Genomic Services for exosome isolation and miRNA isolation. Histopathology was conducted on testes and epididymis of BA treated rats. Tenex BA treatment-related effects, 6 test substrate-related findings included lower epididymis weights, smaller epididymides, and microscopic findings of cellular debris and decreased spermatid cellularity in the epididymis and tubular degeneration/apoptosis and atypical residual bodies in the testes. Tubular degeneration/apoptosis in the test substance-treated group was characterized by vacuolar degeneration of spermatocytes and spermatids. Urine exosomes and miRNA were isolated with QIAseq 52 Spike-ins through exoRNeasy. miRNA sequencing was performed using an Illumina NextSeq 500. The miRNA library preparation was completed using the QIAseq miRNA Library Kit. Several miRNAs were identified in rat urine as potential biomarkers for BA related effects on male reproductive system: miR-34c-5p, miR-449a-5p and miR-122-5p were decreased in BA treated rats compared to untreated controls. These miRNAs have also been identified to be decreased in humans with sertoli cell related spermatogenic failure. BA has been shown to affect Sertoli Cells in the testes of rats. Additionally, let-7p (decreased), miR-141-3p (increased) and miR21-5p (increased) were differentially expressed in BA treated rats. These miRNAs have been identified as potential biomarkers for human male non-obstructive azospermia. Also, miR-27b-3p levels decreased in BA treated rats shown to be associated with asthenozoospermia in humans. These results provide the basis for the potential application of miRNAs as biomarkers for BA related testicular toxicity and highlight the value of urine exosome miRNA NGS as a discovery tool.

1470 Transient Alanine Aminotransferase Increases following Acetaminophen Treatment in Rats
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A persistent concern in pharmaceutical development is the appropriate response to serum alanine aminotransferase (ALT) increases seen in patients during clinical trials. While ALT increases can portend serious liver injury, it is also well known that for certain drugs, such increases in clinical settings reverse with continued treatment, with no further evidence of liver injury. This well-documented phenomenon is often referred to as adaptation. A means to distinguish those ALT increases that will resolve from those that do not is needed. We report here a pilot animal model of BTALT increases. Sprague-Dawley rats were given daily oral doses of 1.0, 1.5 and 2.0 g/kg acetaminophen (APAP) and sacrificed after 8 days of treatment. Tail bleeds were conducted at days 1, 3, 6 and 7 of treatment. ALT increases were seen at day 3 in APAP treated rats. Strikingly, 1) these increases were not seen in all treated animals and the extent varied greatly from animal to animal, 2) all ALT increases returned to baseline by day 6, 3) the increases were not dose dependent, 4) by contrast, no increases in total bilirubin were seen, 5) while minimal to mild centrilobular necrosis was observed at day 8.
for most of the treated animals, its grade was not related to the extent of ALT increase. This represents the first report of transient ALT increases in a rodent model and offers the possibility for discovery of biomarkers of adaptation.

1471 Application of Magnetic Carbon Nanotubes Facilitated Dispersive Micro Solid Phase Extraction of the Cyanide Metabolite (2-Aminothiazoline-4-Carboxylic Acid) in Biological Samples

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The purpose of this presentation is to familiarize the audience with the application of magnetic carbon nanotubes facilitated dispersive micro solid phase extraction (Mag-CNT/d-µSPE) for the minor metabolite of cyanide, 2-aminothiazoline-4-carboxylic acid (ATCA), to biological samples. Confirmation of cyanide exposure by direct detection of cyanide in biological samples may be subjected to interpretation challenge due to its reactive and unstable nature. The concentration of cyanide in biological samples could fluctuate under improper storage conditions, which also increases the difficulties to associate cyanide concentration level in biological samples for cyanide exposure. The detection of biomarkers for cyanide, such as ATCA, was suggested to be an alternative approach to confirm cyanide exposure. In this research, Mag-CNT/d-µSPE was developed to extract ATCA from synthetic urine and bovine blood samples. Briefly, 2 mg of Mag-CNT was added to 100 μL of ATCA-spiked synthetic urine or protein-precipitated bovine blood. The samples were acidified with hydrochloric acid, vortex, and sonicated for 10 min. The Mag-CNT were isolated and the supernatants were decanted. The ATCA on the Mag-CNT was desorbed with 150μL of 5%(v/v) NH4OH/water with sonication. The desorbed supernatants were transferred to separate tubes, dried under vacuum at 65°C and derivatized with N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and subjected to GC/MS analysis. Method parameters, including calibration model, limit of detection (LOD) and quantitation (LOQ), bias, precision, carryover and interference study, were tested for both biological samples. The Mag-CNT/d-µSPE for urine samples demonstrated a satisfactory bias and precision within ± 20% at the low, medium, and high concentration levels. The LOD and LOQ for the urine samples were 15 and 30 ng/mL respectively with an average R2 value of 0.9985. No carryover or interference peaks were observed at the retention time of ATCA in all tested samples. Sample clean up prior to Mag-CNT/d-µSPE may be required for bovine blood due to complex matrix effects.

1472 Temporal microRNA Analysis in Multiple Brain Regions following Soman Exposure in Rats

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Soman is a potent organophosphate acetylcholinesterase inhibitor that results in increased levels of acetylcholine and leads to a cholinergic crisis upon exposure to high doses. This cholinergic crisis induces convulsions and seizures and can result in death if left untreated. Understanding the effects of soman is vital to developing a method of diagnosis and treatment for exposure. Acute and chronic effects of soman exposure are an increasing concern for military and civilian populations. The functional alterations that arise from exposure are associated with dysregulation of complex gene networks, and growing evidence implicates microRNAs as key regulators of these networks. In this study, we examined time and brain region-dependent changes in microRNA expression profiles associated with soman exposed seizure activity in rats. Adult male Sprague-Dawley rats were exposed subcutaneously to soman and heat, kidney, liver, lungs, spleen, and brain regions, including amygdala, hippocampus, hypothalamus, piniform, medial prefrontal cortex, parietal cortex and thalamus were collected after 72 hrs and 90 day exposure. RNA was extracted from each tissue and small RNA was sequenced using next generation sequencing on illumina platform. Initial data from piniform cortex demonstrate significant differences in expression patterns between animals that seized and those that didn’t seize. The miRNAs expression changes across brain tissue types showed hypomethylated to have a quite distinct response from other regions. The results demonstrate that soman exposure results in dynamic and complex temporal changes in microRNA-mRNA gene network structure. Ongoing work will fully characterize the miRNA genes network.

1473 Organophosphate Exposure of Aircraft Maintainers

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Air Force aircraft maintainers have been shown to come into contact with products containing organophosphates, primarily triecyl phosphate (TCP), tributyl phosphate (TBP), and triphenyl phosphate (TPP). These chemicals are often found in aircraft hydraulic fluids and turbine oils. This is particularly troublesome because these chemicals are cholinesterase inhibitors, which are believed to cause behavioral and neurological symptoms. Such symptoms include personality change, mood destabilization, suicidal thought, and memory and attention impairment. In order to assess aircraft maintainers’ exposure to these organophosphates, blood samples were collected before and immediately after volunteers’ shifts. Analyzing acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibition is believed to be an efficient method to measure the exposure to all organophosphates. Because acetylcholine is known to reside in the red blood cells (RBC), the RBC were isolated from the blood samples provided by the volunteers to assess AChE activity. Similarly, the plasma was isolated to evaluate BuChE activity. These separated samples were then analyzed with an Ellman assay to determine the levels of AChE and BuChE inhibition. Fortunately, only two of 83 volunteers showed concerning levels of cholinesterase inhibition. This level was defined as 50% decline of baseline cholinesterase, as this amount of inhibition may coincide with symptoms such as headache, vomiting, and cramping.

1474 Downregulation of Wild-Type Tumour Suppressor Protein in Nigerian E-waste Population: Risk of Disruption of Genome Protection Mechanism

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Informal electronic waste (e-waste) reprocessing in Nigeria is reportedly the highest in Africa, predisposing exposed humans to risk of metal exposure. The management practices in Nigeria are crude and environmentally unsound. Most e-waste-born metal pollutants and metals have been reported to have potentials for metal carcinogenesis by their direct genotoxic effects and disordered metalloregulation of gene repair mechanisms. There is currently dearth of data on predictive biomarkers for evaluation of genotoxicity in the e-waste exposed population in Nigeria. This study aimed to evaluate the status of genotoxic biomarkers: wild-type tumour suppressor protein (wt-p53), DNA repair enzyme 8-oxoguanine-DNA glycosylase (OGG1), and oxidative DNA damage biomarker; 8-hydroxy-2-deoxyguanosine (8-OHdG), and correlate with blood levels of toxic metals and essential metals in 381 e-waste workers, 120 environmental e-waste exposed participants, and 131 age-matched unexposed controls in South-West Nigeria. From the blood samples obtained, Enzyme-Linked Immunosorbent Assay was used to determine genotoxicity biomarkers in serum while Inductively Coupled Plasma-Mass Spectrometry was used to determine essential metal levels in serum and toxic metal levels in whole blood. From the results, we observed significantly raised blood levels of toxic metals, and lower serum levels of Zn, Cu, Se and Co in the e-waste exposed populations. Additionally, there was a repressing of wt-p53 expression, accompanied by the upregulation of 8-OHdG in the e-waste groups. OGG1 activity, was slightly higher in e-waste workers than in controls, but this difference was not significant; in environmentally exposed participants, OGG1 activity, was slightly higher in e-waste workers than in controls, but this difference was not significant; in environmentally exposed participants, OGG1 activity, was significantly higher when compared with the e-waste workers and unexposed controls. These data appear to provide evidence of elevated body burden of genotoxic metals, decreased levels of genome-protective metals and repression of p53 expression coupled with raised oxidative DNA damage activity, all of which appear suggestive of the risk of disruption of genome protection mechanism in the e-waste exposed populations.

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1475 Increased Autoantibodies to Glial Fibrillary Acidic Protein (GFAP) in Plasma of Veterans with Gulf War Illness (GWI)

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Following the 1991-1992 Gulf War, approximately one third of the U.S. troops out of 700,000, complained of chronic symptoms, which is now known as Gulf War Illness (GWI). This illness is characterized by multisymptomatic disorder that consists of joint pain, gastrointestinal problems, memory and difficulty concentrating. In this study, we identified and determined the levels of autoantibodies against two main glial proteins. One is glial fibrillary acidic protein (GFAP), which is an astrocytic protein that mainly contributes to white matter architecture, myelination, maintains the mechanical strength of astrocytes and provides integrity to the blood-brain barrier. The other one is S100B protein, which is expressed primarily in astrocytes, known to stabilize microtubule associated proteins; MAP-2 and Tau. The action of S100B is neurotropic at a nanomolar serum concentration and apoptotic at a micromolar concentration. Acute traumatic brain injury resulting in large destruction of astrocytes leads to massive (50- to 100-fold) release of S100B in serum, whereas levels of S100B in psychiatric disorders were only three times higher in patients compared to the controls. Correlating well with their neuroprotective action, we determined the level of autoantibodies specific to GFAP and S100B using a western blot assay in the plasma of veterans with GWI (n=68) compared to the non-veteran asymptomatic controls (n=26). We observed increased level of autoantibodies specific to GFAP protein in the plasma of veterans with GWI compared to controls. The increase was nearly nine-fold compared to healthy controls for GFAP and the significance was estimated by the Fischer’s exact test to be p-value less than 0.0001, but the autoantibodies specific to S100B showed an insignificant increase compared to the healthy controls consistent with the chronic condition of the GWI. Because of the long lasting nature of LgG, detection of autoantibodies studied would be an indicator of brain injury characteristic of GWI. Conclusively, autoantibodies to GFAP may signify a chronic CNS damage. Supported in part by DOD W81XWH-15-1-0641.

1476 PAH Exposure among Slaughterhouse and Slaughter Slab Workers Who Utilize Scrap Automobile Tires to Singe Meat in Ghana

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In Ghana and many other African countries, it is common practice for slaughterhouse (SLH) and slaughter slab (SLS) workers to utilize scrap automobile tires as fuel to singe the fur of food animal carcasses intended for human consumption. This practice releases many toxic substances including polycyclic aromatic hydrocarbons (PAHs), carbon monoxide (CO), particulate matter, and other toxicants into the air via smoke. PAHs are a group of more than 100 organic compounds that is released during the combustion of tires, coal, oil, gasoline, trash, tobacco, and wood. Exposure to PAHs has been associated with a plethora of health issues such as respiratory impairment; irritation of skin, eyes, and airways; and cancer. Studies have shown that 1-OHP is a reliable biomarker of occupational and residential exposures to PAHs because 1-OHP is a metabolite of pyrene, one of the significant components known to be present in all PAH mixtures (Hu and Hou, 2015; Jongeneelen, 2001). Our previous studies suggested that the level of 1-OHP was differentially accumulated among SLH-SLS operators who use tires as fuel to singe meat; however, the health implications were not evaluated. Therefore, the aim of this study is to assess Kumasi SLS operators’ exposure to PAHs by quantifying their urinary 1-OHP levels using HPLC with fluorescence capability. Urine samples (n=59) were collected from SLH-SLS operators who utilized tires (363.43±61.65 ng/mg crt) compared to production workers (319.75±115.49 ng/mg cort) and those who utilized LPG (210.47±54.17 ng/mg cort). The urinary 1-OHP level of SLH-SLS operators who use tires as fuel to singe meat was significantly higher than workers who used LPG (5.02%). These higher exposure levels indicate potential health risks among the individuals, and results of these data revealed high rate of AFB, exposure in Kumasi slaughterhouse and SLS workers. Studies are on-going to evaluate the associations between maternal and childhood AFB exposure, and the growth and development of young animals.

1477 Aflatoxin Exposure Assessment in Ugandan Mothers and Children

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Aflatoxin B1 (AFB1), a potent mycotoxin produced by Aspergillus flavus and Aspergillus parasiticus, is a ubiquitous food contaminant and a well-known liver carcinogen which has been categorized by IARC as a known human carcinogen (Group 1). Recent evidence has suggested the link between AFB exposure, exposure and impairments in growth and development of young animals and children, and AFB exposure may be one of major risk factors for childhood stunting in many low- and middle-income nations. In this study, AFB exposure was assessed via analysis of a validated expression biomarker, serum AFB1-lysine adduct, using HPLC-FLD based method for a total of 3326 serum samples (n=1551 from children and n=1774 from mothers), all collected on the fourth quadrant of 2017 for a birth cohort study conducted in Uganda. The overall detection rate of serum AFB1-lysine adduct was 69.67%, 82.49% for the children samples, and 95.89% for the mothers samples. The median level of serum AFB1-lysine for all collected samples was 2.63 pg/mg albumin, with first and third quartile at 1.12 and 6.80 pg/mg albumin. The overall geometric mean was 3.06 pg/mg albumin (95% CI: 2.92, 3.21). The mothers generally had higher levels of serum AFB1-lysine adduct than children (p<0.0001). The median level in the mother samples is 4.33 pg/mg albumin, with first and third quartile at 1.98 and 10.59 pg/mg albumin. The geometric mean is 4.80 pg/mg albumin (95% CI: 4.52, 5.11). The median level in children samples 1.39 with first and third quartile at 0.76 and 2.98. The geometric mean is 1.68 pg/mg albumin (95% CI: 1.58, 1.78). Significantly high levels of AFB1-lysine adduct (>50 pg/mg albumin) were found in 102 of the 3326 analyzed samples (3.07%), with 13 from the children group (0.84%) and 89 from mother group (5.02%). These higher exposure levels indicate potential health risks among this individuals, and results of these data revealed high rate of AFB exposure in Uganda mothers and children. Studies are on-going to evaluate the associations between maternal and childhood AFB exposure, AFB exposure and the growth and development of young animals.

1478 Use of Neopterin as a Biomarker of Immune Activation in Preclinical Species

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Neopterin is a pteridine metabolite that is produced following activation of macrophages and dendritic cells in humans and in other species. While the levels of neopterin in humans is characterized, the potential to use the measurement of neopterin as a biomarker of immune activation in preclinical species is not known. Here we describe the development of a sensitive LC-MS/MS method to quantify neopterin in blood samples. The validity of using neopterin to measure immune activation was demonstrated by ex vivo stimulation of blood samples. For the LC-MS/MS analysis, both control and spiked samples were processed by spiking with internal standard diluted in organic mobile phase followed by solid phase extraction, and analysis. The results of this experiment demonstrate increased neopterin in stimulated blood compared to unstimulated samples. The optimized method was successfully applied to samples from toxicological studies with preclinical species in which immune activation was anticipated. This work demonstrates the potential utility of neopterin as a biomarker candidate of immune activation with application to multiple preclinical species.

1479 Circulating Cell-Free DNA (ccfDNA) as a Liquid Biopsy Tool in Rodent Toxicology


Short fragment extracellular DNA (~180bp) normally circulates in blood at low levels. Cell death in tissues (normal turnover) is a primary source of circulating, cell free DNA (ccfDNA), both of nuclear and mitochondrial origin. CcfDNA was first described in the 1940’s but more recently its significance in diagnosis, staging and biomarker discovery has gained much attention as a point-of-care, minimally invasive form of liquid biopsy in a variety of clinical conditions such as cancer, fetal aneuploidy disorders, obesity, autoimmune disorders and organ transplantation. To date, few studies have investigated the use of ccfDNA in environmental health science. We explored the utility of ccfDNA in biomarker development for chemical toxicity and exposure in experimental animals by using successful examples of ccfDNA analysis of adult and neonatal rodents. Purified ccfDNA yields ranged from 4.8 ng per sample and displayed the characteristic DNA nucleosome fragmentation pattern of
CFT073 strain. This particular sin

1482 Evaluation of miRNAs as Biomarkers for MerTK-Mediated Retinal Degeneration
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The roles of MerTK in immunity and cancer has made it a potential therapeutic target. MERTK also has an essential role in specialized phagocytosis of apoptotic cells, specifically in the retina and in various cancer cell lines. These functions are critical in the eye upon waking. MerTK mediates effectorcytic activity of the retinal pigmented epithelium, which is essential in clearing spent photoreceptors in the retina. Therefore, there is potential for retinal toxicity if MERTK is inhibited in the retina and identification of a biomarker for potential retinal toxicity would be advantageous for the therapeutics that block MerTK signaling. The miR182/183/96 cluster has been implicated in various retinal disorders including retinitis pigmentosa. In this study, we compared the expression levels of miR96, miR182 and miR183 in serum samples of wild-type, MERTK homozygous and heterozygous mice of at least 2 months of age. Retinas were evaluated histologically by thin plastic section. Moderate upregulation of miR182 and miR96 was observed in the homozygous knockout animals compared to wild-type mice. Very little to no change in expression of these microRNAs was observed in MerTK heterozygous mice. These results correlate with histological findings indicating a complete loss of photoreceptors and depletions of the outer nuclear and outer plexiform layers in the retinas of homozygous mice. No changes were observed in the retinas of heterozygous mice. The results of these studies suggest miR182 and miR96 may be promising biomarkers of MerTK-mediated retinal toxicity.

1483 Optical Densities as Measures to Assess In Vitro Therapeutic Equivalences, Antimicrobial Susceptibilities, Resistances, Toxicities against Escherichia coli CFT073
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There is an important requirement to develop generic evaluate and compare antimicrobial drugs activities and toxicities because of their demand to combat increased resistance of pathogenic bacteria to current antibiotics and to minimize toxicity levels. In vitro therapeutic equivalence studies of generic antimicrobials can add value to pharmaceutical equivalence studies and also can facilitate regulatory decision making for combination therapies. We used 24 kinetic measurements of bacterial growth in a standard, clear, 96-well plate, with a standard absorbance microplate reader to enable screening of three antimicrobial drugs in and their potential for delaying the growth of Escherichia coli CFT073 strain. This particular single strain was used to model uncomplicated urinary tract infection (UTI) in vitro. Three antibiotics, namely Ampicillin, Ciprofloxacin Hydrochloride and, Fosfomycin Disodium Salt, their dual and triple combinations were evaluated independently and in combinations by means of their consistently varied minimum inhibitory concentrations (MICs) in each well by maintaining constant density of E.coli. E. coli CFT073 at about the density of 50,000/well. These chemical-biology studies enabled not only the determination of in vitro therapeutic equivalences of antibiotics, but also their independent and combined influences on bacterial growth. Two to five times of order differences are consistently evident between 0 times MIC, 3 times MIC, 10 times MIC and 30 times MIC concentrations of the antibiotics. In conclusion, the generic antibiotic products can be quickly compared with their innovator drugs to predict their therapeutic equivalences, toxicities and to determine their resistance potentials.

1484 Comparison of Lactate Concentration with Different Anticoagulant

Lactate is organic anion of buffered lactic acid, the end product of glycolysis pathway and an indicator of the oxygen-containing state of cell tissue. Blood samples for quantitative lactate analysis usually use sodium fluoride/potassium oxalate as anticoagulant in order to prevent in vitro glycolysis and increased lactate production. However, sometimes the anticoagulant...
EDTA-K2 may be used to collect blood samples due to the reason of some compounds’ nature. The sodium fluoride is not available. The blood samples were collected into tubes with anticoagulant sodium fluoride and EDTA-K2, respectively. The lactate concentration was tested for 1, 2, 4, and 6 hours after blood collection and recorded as time 1, 2, 4, and 6, respectively. The parallel comparison of the same time slot and species was conducted between two anticoagulants. The statistical analysis of T-test and Wilcoxon were done based on homogeneity of variance of test data. For species monkey, there is no significant difference for the lactate concentration between tubes with sodium fluoride and EDTA-K2 at any time points (time 1, 2, 4 and 6) (p<0.05). For species dog, there is no significant difference for the lactate concentration between with sodium fluoride and EDTA-K2 at time 1 hour (p<0.05), but significant differences were observed at time 2, 4 and 6 (p<0.05). The test results showed that there was no obvious increase of lactate in species monkey under anticoagulant EDTA-K2 conditions. However, the concentration of lactate had mildly increased in species dog containing anticoagulant EDTA-K2. Our research indicates that EDTA-K2 is recommended as the anticoagulant for lactate analysis in monkeys but does not apply to the dogs.

1484a Performance of Neurofilament Light in Rat as a Translational Biomarker of Central and Peripheral Nervous System Toxicity
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Target organ toxicity is often a reason for compound attrition during drug development. Use of safety biomarkers can help to monitor such toxicities early and efficiently in preclinical species, and allow translation to early clinical trials while ensuring patient safety. While accessible biomarkers for end-organ toxicities like liver, kidney or muscle are widely available across preclinical species and human, others remain in blind spots for the central (CNS) and peripheral (PNS) nervous system. Neurofilament light (NF-L) is an emerging biomarker that has been studied recently in humans for numerous neurological disorders including traumatic brain injury, amyotrophic lateral sclerosis, multiple sclerosis and Huntington’s disease. Here we evaluated the performance of NF-L (measured by Quanterix NF-Light® assay) in monitoring drug-induced CNS or PNS toxicity by using plasma, serum and CSF samples from in vivo rat studies with model CNS/PNS toxicants, as well as with toxicants not causing neuronal damage to address specificity. Representative toxicants included chlorpropionic acid, hexachlorophene, 3-nitropropionic acid, d-aminomethylamine, dexamethasone, vincristine, acrylamide and two MoV compounds. A standard histomorphologic assessment based on hematoxylin and eosin stained tissue sections of routine nervous tissue was conducted to diagnose nervous system injuries. In all evaluated CNS/PNS studies with the exception of dioxin/epoxide, NF-L increased in a dose- or time-dependent manner correlating to neuronal degeneration/necrosis in CNS or to axonal degeneration in peripheral nerves. In some cases, NF-L increases were observed in studies treated with CNS/PNS toxicants but without corresponding histomorphological changes, while no NF-L changes were noted in specificity studies presenting with severe liver, kidney, muscle or bone marrow toxicity. These results suggest higher sensitivity of NF-L as an in vivo biomarker in peripheral nerves compared to CNS. In plasma results of CNS datasets, a standard histomorphologic assessment on routine nervous tissue in detecting nervous system injuries. In conclusion, NF-L is an emerging sensitive and specific translational biomarker for use in rat, where it can be used to monitor for nervous system injuries. In conclusion, NF-L is an emerging sensitive and specific translational biomarker for use in rat, where it can be used to monitor for nervous system injuries.

1485 The Effect of Phthalates on the Population of Primaldial Germ Cells of Xenopus laevis before and after Gastrulation
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Phthalates are a class of chemicals that are used in personal care products, medical devices, and many more industrial products. The biological effects of phthalates have been studied because they are ubiquitous and are linked to adverse human reproductive outcomes, such as fertility complications, decreased anogenital distance, and reduced testosterone levels. The effects of exposure to such chemicals are studied in later stages of development. However, in order to understand the outcomes of phthalate exposure on the reproductive system of an organism, it is important to study these effects at earlier stages of development and monitor the changes. Hence, it was hypothesized that a phthalate mixture decreases the number of primordial germ cells (cells that later become gametes) in early embryogenesis prior to zygotic genome activation. To test the above hypothesis, Xenopus laevis embryos were cultured and exposed to a relevant phthalate mixture at specific developmental stages which are before and after gastrulation. The phthalate mixture was based on the exposure levels found in urine metabolites of pregnant women in the Illinois area. It was composed of 21% di(2-ethylhexyl) phthalate (DEHP), 35% diethyl phthalate (DEP), 15% dibutyl phthalate (DBP), 8% di-isobutyl phthalate (DiBP), 5% benzyl butyl phthalate (BBzP), and 15% di-isononyl phthalate (DiNP) for 48 hours. A four-day assay referred to the whole embryo toxicity assay (WETA) was conducted using primary germ cell biomarker called xpat probe to locate the primordial germ cells. Exposure to the various phthalate mixture concentrations (1 µg/ml, 10 µg/ml, and 100 µg/ml) was shown not to affect the embryo viability within 72 hours. However, a significant reduction of primordial germ cells was shown in embryos treated before gastrulation of 10 µg/ml (p<0.0001) and 100 µg/ml (p<0.0004) when compared to the control group. Embryos exposed after gastrulation did not show any significant change in the primordial germ cell abundance in any of the treatment group. The data indicate that exposure to a biologically relevant phthalate mixture reduces significantly primordial germ cells before gastrulation in the early embryonic development of an organism where the zygotic genome is silent whereas, after gastrulation, the zygotic genome becomes active. More studies should be done to assess the link between zygotic genome activation and endocrine disrupting chemicals.

1486 Iodoacetic Acid Inhibits Follicle Growth and Alters Expression of Genes That Regulate Apoptosis and the Cell Cycle in Mouse Ovarian Follicles
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Water disinfection was one of the most important public health advances of the last century because it greatly reduced the incidence of waterborne diseases. The reaction between disinfection products and organic and inorganic matter in source water generates the unintended formation of water disinfection by-products (DBPs). Exposure to DBPs has been associated with adverse developmental effects and it causes cytotoxicity and genotoxicity in mammalian cells. Further, iodoacetic acid (IAA), an unregulated DBP, was shown to be a reproductive toxicant in vitro. However, its effects on the ovary are not well known. This study was designed to determine whether IAA exposure affects ovarian follicle growth and expression of genes that regulate apoptosis and the cell cycle. Ovaries were collected from CD-1 mice (32 to 42 days old). Antral follicles (220 to 400 µm) were dissected from the ovaries and placed individually in 96-well culture plates. The follicles were treated with either 0.075% dimethyl sulfoxide (DMSO; vehicle control) or IAA (2, 5, 10, or 15 µM). The follicles were cultured for 96 h and follicle growth was measured every 24 h. After 96 h of culture, follicles were collected and snap-frozen at -80°C until RNA extraction. RNA was extracted, reverse transcribed, and subjected to quantitative polymerase chain reaction (qPCR) to analyze expression levels of apoptosis regulators (Bax and Bcl2) and cell cycle regulators (Ccn2, Ccn1, Ccnb1, Ccn2, Cd4 and Cdkn1a). IAA exposure (10 and 15 µM) significantly decreased follicle growth compared to controls, beginning at 72 h and continuing through 96 h of culture. Further, IAA exposure decreased expression of the cell cycle regulators Ccn2 (10 and 15 µM) and Ccn1 (15 µM) and it (10 and 15 µM) decreased expression of the anti-apoptotic factor Bcl2. In addition, IAA exposure (15 µM) increased expression of the pro-apoptotic factor Bax and the cell cycle regulator Cdk4 and it (10 and 15 µM) increased expression of the cell cycle inhibitor Cdkn1a. Collectively, these data show that IAA exposure inhibits follicle growth and upregulates pro-apoptotic factors and cell cycle inhibitors, whereas it downregulates anti-apoptotic factors and some cell cycle regulators, thus interfering with apoptosis and proliferation in ovarian follicles. Supported by NIH R21 E0528963 and NIH T32 E0507326.

1487 Metabolism of an Environmentally Relevant Phtalate Mixture in Mouse Ovaries

Phthalates are synthetic chemicals commonly used as additives in consumer products with widespread human exposure. While phthalates are manufactured as diesters, they are hydrolyzed in the environment and in the body to monoesters that may be more toxic than the parent compounds. Individual phthalates have been shown to be reproductive toxicants, but few studies have examined the toxicity of mixtures of phthalates. This study tested the hypothesis that neonatal and adult mouse ovariess are able to metabolize an environmentally relevant mixture of phthalates. Whole neonatal ovariess (postnatal day 4) and adult female ovariess from CD-1 mice were cultured in media treated with DMSO (vehicle control) or 0.1, 1, 10, or 100 µg/mL of a mixture composed of 35% diethyl phthalate (DEP), 21% di(2-ethylhexyl) phthalal
Phthalates are chemicals used as plasticizers, which increase flexibility of plastic, thus causing them to leach. Due to leaching humans are constantly exposed to phthalates at all ages. Phthalates are linked to health problems such as infertility due to interference with hormones and have been shown to affect preimplantation embryo development. Studies have shown that exposure to a single phthalate effects embryo development. However, there is limited data on the effects of exposure to relevant phthalate mixtures (PM) on preimplantation embryos (PE). The PM used in this study is composed of DEP (35%), DEHP (21%), DBP (15%), DINP (15%), DIBP (8%) and BBzP (5%). These phthalates and percentages are based from measurements obtained from pregnant women in the UIUC iKis study. The hypothesis was that environmentally relevant PM will interfere with PE development causing cellular arrest and increased fragmentation. Also, that the PM will decrease blastocyst cells within inner cell mass (ICM) and trophectoderm (TE). Female CD-1 mice at 39 days of age were superovulated then mated with C57BL6/B males. Zygotes were collected and distributed into one of six treatment groups, control, DMSO, 0.001 μg/mL, 0.01 μg/mL, 0.1 μg/mL, and 1 μg/mL. To determine whether exposure to the PM affects development, embryos were observed at the 2-cell, 8-cell, morula, blastocyst, and hatched blastocyst stages. In addition, the presence of fragmentation was assessed to determine quality. To determine whether the PM altered blastocyst cells within the ICM and TE, hatched blastocysts underwent immunofluorescence staining with Oct-4 to observe the ICM and Cdx-2 to observe the TE, cells were counted using Imaris Software. Exposure to the PM decreased the number of embryos that developed to the hatched blastocyst stage (0.001 μg/mL, 0.1 μg/mL, 1 μg/mL) and increased the percentage of fragmentation in treatment groups 0.01 μg/mL and 0.1 μg/mL. Although our results showed a decrease in embryo development when exposed to the PM, a small percentage were able to hatch. If staining showed that there was a decrease in the total number of cells within the ICM of embryos cultured in 1 μg/mL. Our results suggest that exposure to a relevant PM effects PE development, increases fragmentation, decreases the cell population within the ICM thereby, reducing the viability of PE.
Di(2-ethylhexyl) phthalate (DEHP) is used as a plasticizer in several consumer products and readily leaches from these products, leading to daily human exposure. Of concern, DEHP has been shown to have endocrine disrupting capabilities, and compounding this concern is a lack of knowledge on the effects of DEHP on fertility. Some manufacturers have elected to replace DEHP with other plasticizers. However, often less is known about these DEHP alternatives compared to DEHP itself. Diisononyl phthalate (DiNP) is one such understudied replacement. Thus, this study tested the hypothesis that adult exposure to DEHP or DiNP negatively affects female fertility. To test this hypothesis, adult female CD-1 mice (39–40 days) were orally dosed with vehicle control (corn oil), DEHP (20 or 200 µg/kg/day or 20 or 200 mg/kg/day), or DiNP (20 or 100 µg/kg/day or 20 or 200 mg/kg/day) for 10 days. Mice were euthanized in diestrus immediately following dosing or three or six months post-dosing. Estradiol, progesterone, and testosterone were analyzed in sera via enzyme-linked immunosorbent assays. A cohort of mice were bred immediately post-dosing and at three and six months post-dosing. Immediately post-dosing, DEHP and DiNP decreased testosterone (20 mg/kg/day DEHP, 100 µg/kg/day and 200 mg/kg/day DiNP, p ≤ 0.05) and estradiol (20 µg/kg/day DEHP, p = 0.055, 20 mg/kg/day DEHP, 100 µg/kg/day and 200 mg/kg/day DiNP, p ≤ 0.05) and increased progesterone levels (200 mg/kg/day DEHP, p ≤ 0.05, and 20 mg/kg/day DiNP, p = 0.0998). At three months post-dosing, DiNP decreased progesterone (100 µg/kg/day, p ≤ 0.05), increased estradiol (200 mg/kg/day, p = 0.083), and decreased progesterone levels (20 µg/kg/day, p = 0.082, 100 µg/kg/day, p ≤ 0.05). At six months post-dosing, DiNP decreased testosterone (100 µg/kg/day, p ≤ 0.05) and DEHP increased progesterone levels (200 µg/kg/day, p ≤ 0.05). Immediately post-dosing, no treatments affected male reproductive fertility. However, at three months post-dosing, DEHP and DiNP decreased the ability of females to become pregnant (20 µg/kg/day, p ≤ 0.05). At six months post-dosing, DEHP increased pregnancy loss (200 µg/kg/day, p ≤ 0.05). These data show that acute exposures to DEHP and a common DEHP replacement, DiNP, have long-lasting impacts on hormone levels and fertility for several months after exposure. Supported by R56 ES 025147 (JAF), R01 ES 028661 (JAF), and T32 ES 007326 (CC).
1495 Ancestral Exposure to Di(2-Ethylhexyl) Phthalate Disrupts Gene Expression and Pathways Critical for Mouse Ovarian Function in Three Generations

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Di(2-ethylhexyl) phthalate (DEHP) is plasticizer found in polyvinyl chloride. DEHP can leach from these products and is a known endocrine disruptor. Previously we showed that prenatal DEHP exposure disrupts ovarian functions and female fertility in a transgenerational manner. However, little is known about the mechanism by which prenatal exposure to DEHP impacts ovarian functions and fertility in female mice across three generations. Therefore, this study investigated the changes in the gene expression in the ovaries from mice prenatally and ancestrally exposed to DEHP in the F1, F2, and F3 generations. Pregnant CD-1 mice (F0 generation) were orally dosed with tocopherol-ol-stripped corn oil (vehicle control) or DEHP (20, 200 µg/kg/day, 500, or 750 mg/kg/day) daily from gestation day 10.5 until birth (7-28 dams/treatment group). Pups born to these dams were considered the F1 generation. F1 females were mated with untreated males to obtain the F2 generation, and F2 females were mated with untreated males to produce the F3 generation. On postnatal day 21, female pups from each generation were euthanized and their ovaries were removed and frozen in liquid nitrogen and subjected to qPCR to quantify the mRNA expression of the phosphoinositide 3-kinase (PI3K) pathway, cell cycle regulators, steroid hormone receptors, insulin-like growth factors, and DNA methyltransferases. The results indicate that in the F1 generation, prenatal exposure to DEHP increased expression of PI3K factors and insulin-like growth factors. Further, it dysregulated expression of cell cycle regulators and steroid hormone receptors. In the F2 generation, prenatal exposure to DEHP dysregulated expression of PI3K factors and cell cycle regulators. It also decreased expression of steroid hormone receptors. In the F3 generation, ancestral exposure to DEHP decreased expression of PI3K factors, cell cycle regulators, steroid hormone receptors, insulin-like growth factors, and DNA methyltransferase. Collectively, these data indicate that prenatal and ancestral DEHP exposure causes transgenerational and trans-generational changes in the expression of genes in pathways that are critical for normal ovarian functions. Further, these data suggest that DEHP-induced changes in expression may influence DNA methylation. Supported by NIH PO1 ES022848, US EPA RD-83459301, T32 ES007326, and the Billie A. Field Fellowship.

1496 Prenatal Exposure to a Phthalate Mixture Changes Uterine Morphology and Differentiation in Mice in Multiple Generations

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Phthalates are found in plastic containers, children’s toys, and medical supplies. They are endocrine disruptors and exposure to them leads to human health risks. Animal studies show that they disrupt ovarian development, reduce fertility and promote reproductive diseases in females. Most studies focus on a single phthalate, but humans are exposed to phthalate mixture (PM) daily. We hypothesized prenatal exposure to relevant doses of PM may change uterine morphology and function in mice for generations. The PM was based on urine phthalate composition of pregnant women: 35% diethyl phthalate, 21% di(2-ethylhexyl) phthalate, 15% dibutyl phthalate, 15% diisononyl phthalate, 8% diisobutyl phthalate, and 5% benzylbutyl phthalate. CD-1 dams were orally dosed with vehicle, or PM at 20 and 200 µg/kg/day, 200 µg/kg/day from pregnancy day 10.5 to birth. Uteri of F1, F2 and F3 female offspring (n=6-8/group) were collected, weighed, and processed for histological evaluation at 12 month. Samples were stained for H&E and immunohistochemistry for smooth muscle α-actin (SMA) and Ki67. Slides were scanned using Nanozoomer. Thickness of myometrial layers, size of endometrium, numbers of endometrial glands and proliferating cells in the luminal (LE) and glandular epithelium (GE) were measured. Data were analyzed by one way ANOVAs with multiple comparison against controls. No changes in size of endometrium and thickness of myometrial layers were similar in F1, F2 or F3 mice. We saw an increase in LE proliferation in the highest groups and a decrease in GE proliferation in the 200 mg/kg/day group in F2. In F1 500 mg/kg/day group, F2 200 µg/kg/day, 200 and 500 mg/kg/day groups, and F3 20 µg/kg/day, 200 and 500 mg/kg/day groups, we saw increased SMA expression in the endometrium, indicating presence of myofibroblasts and a fibrotic response. Glycosed cysts, large dilated glands and areas of epithelial hyperplasia were more prevalent in treated mice. These changes are linked to uterine pathologies such as endometritis and cystic hyperplasia in women. In conclusion, prenatal exposure to PM increases uterine morphological abnormalities of F1, F2 and F3 mice as they age, reflecting changes in the normal pattern of proliferation vs differentiation and inflammatory response. Funded by NIH R01ES022848 (JAF), NIH R21ES026389 (RAN) and Environmental Toxicology Fellowship (CZ).

1497 Effects of In Vitro Exposure to Di-n-butyl Phthalate and Mono-n-butyl Phthalate on Early Embryo Viability and Development

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Di-n-butyl phthalate (DBP) is found in many consumer products including: plastics, beauty products, insecticides, medical devices, and oral medications. Phthalate exposure in women has been associated with decreased oocyte retrieval during in vitro fertilization protocols and greater incidence of early pregnancy loss. DBP is rapidly metabolized by non-specific lipases into mono-n-butyl phthalate (MBP) in the body. Previous studies have reported that MBP levels in the follicular fluid of women range from 1.08-2.63 ng/mL, current studies have not evaluated the effects of MBP at these concentrations on embryo development. This study was designed to determine whether environmentally relevant in vitro exposures to DBP and MBP affect development and ATP concentration in early mouse embryos. CD-1 females (35-50 days old, n = 10 mice per time point) were given intra-peritoneal injections of PMSG (5 IU) followed by hCG (5 IU) 48 hours later. Oviducts from each animal were collected at 24 hours post-breeding and embryos recovered (n = 13-51 embryos/sample) were subjected to qPCR to quantify the mRNA expression of the phosphoinositide 3-kinase (PI3K) pathway, cell cycle regulators, steroid hormone receptors, insulin-like growth factors, and DNA methyltransferase. The results indicate that in the F1 generation, prenatal exposure to DBP increased expression of PI3K factors and cell cycle regulators. It also decreased expression of steroid hormone receptors. In the F3 generation, ancestral exposure to DBP decreased expression of PI3K factors, cell cycle regulators, steroid hormone receptors, insulin-like growth factors, and DNA methyltransferase. Collectively, these data indicate that prenatal and ancestral DBP exposure causes transgenerational and trans-generational changes in the expression of genes in pathways that are critical for normal ovarian functions. Further, these data suggest that DBP-induced changes in expression may influence DNA methylation. Supported by NIH P01 ES026998 (ZRC).

1498 Measurements of Mono-n-butyl Phthalate in the Tissues of Cycling Adult CD-1 Female Mice after the Oral Administration of Di-n-butyl Phthalate

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Di-n-butyl phthalate (DBP) is an endocrine disruptor commonly used worldwide as a plasticizer or solvent in many consumer products such as infant care, personal, and cosmetic products. Exposure to endocrine disruptors such as DBP is postulated to cause developmental and reproductive toxicity. While various studies utilizing mice have shown that DBP causes health problems, no quantitative evidence of the active metabolite of DBP, mono-n-butyl phthalate (MBP), in the tissues of adult cycling mice after oral exposure of DBP currently exists. Therefore, to investigate whether there are measurable MBP levels within the tissues of mice treated with DBP, we utilized a highly sensitive liquid chromatography/tandem mass spectrometry assay to determine MBP levels in liver, serum, and ovary of adult CD-1 female mice after one oral dose of DBP. We pipet-fed adult CD-1 mice (N=5 per treatment/time point) with tocopherol-stripped corn oil (vehicle) or one dose of DBP (1000 mg/kg) and collected liver, ovary, and serum at 2, 6, 12, and 24 hours after treatment. In mice treated with vehicle, background MBP levels were detected in serum (0.109±0.07 µg/ml) and liver (0.02±0.009 µg/g), but not in ovaries after 6 hours. In mice treated with DBP at 1000 mg/kg, MBP was detected in serum (15.74±2.46 µg/ml), liver (2.78±0.43 µg/g), and ovary (2.03±0.53 µg/g) at 2 hours, while MBP treated embryos showed a significant decrease in ATP production at 24 hours (DMSO: 193.27±26.23, n=5 wells,77 embryos, MBP: 69.77±15.16, n=4 wells, 49 embryos; p<0.05). ATP concentration did not differ between treatment groups at 72 hours when compared with control. These findings suggest that exposure to environmentally relevant concentrations of MBP do not affect early development but reduce ATP concentration in early embryos. This work was supported by NIH grants R01ES026998 (ZRC).
results in direct effects on the ovary. Future studies will be aimed at evaluating the distribution of MBP to various reproductive tissues following repeated oral dosing with various DBP dosages. This work was supported by NIAMS grant R01026998-01A1 (ZRC).

1499 Di(2-Ethylhexyl) Phthalate (DEHP) Decreases the Extrusion of the First Polar Body in Murine Oocytes at Different Reproductive Ages

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Di-(2-ethylhexyl) phthalate (DEHP) is considered an endocrine disruptor that may impair zygote formation and survival of the embryo. These effects may be exacerbated in women that become pregnant at advanced ages due to reduction in the number of oocytes undergoing maturation, evidenced by the extrusion of first polar body. However, it is unknown whether the detrimental effects in advanced ages are exacerbated by DEHP exposure. This study evaluated the effect of DEHP exposure on the oocyte maturation in mice at different reproductive ages. Female CD-1 mice (3–4 weeks-old) were orally exposed to DEHP 20, 200 and 2000 μg/kg/day for 30, 60 and 120 days. The control group was administered with tocopherol-free mineral oil. Estrous cyclicity was evaluated daily by vaginal cytology. Following dosing, females were superovulated by injecting IP 5 IU of pregnant mare serum gonadotropin (PMSG), followed by 5 IU of human chorionic gonadotropin (hCG) at 48 h later. 18 h after hCG injection, females were euthanized by cervical dislocation, and the cumulus-oocyte complexes (COCs) were obtained from the oviducts to strip the cumulus cells. The oocytes were stained with DAPI and anti-a-tubulin, and then they were classified according to the degree of maturation, such as germinal vesicle stage (GV), metaphase I (MI), and metaphase II (MII). Significant differences in decreased number of COCs were observed in the 2000 μg/kg/day DEHP group treated for 30, 60, and 120 days (≈ 35%, 53%, and 75% reduction, respectively) compared to control. Similarly, the number of oocytes having polar body extrusion (MII) significantly decreased (≈ 55%, 46%, 62%) compared to control. These data suggest that DEHP impairs the resumption of meiosis in a time-dependent manner as mice age.

1500 Examining the Impact of DEHP Exposure via Food on Reproductive Function in Adult Men

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Di-2-ethylhexyl phthalate (DEHP) is a widely used plasticizer that has been shown to exhibit endocrine-disrupting effects and reproductive toxicity. DEHP is found in various plastics including PVC-containing equipment, vinyl flooring, DEHP’s toxicological protective equipment, and food packaging. Because DEHP is not chemically bound to these plastics, it commonly leaches into the environment. It is estimated that approximately 90 percent of human exposure to DEHP is via food consumption, however occupational exposures are also commonly observed. Animal studies have shown that developmental exposures to DEHP can negatively impact the developing male reproductive system. However, few studies examine exposure and potential effects of DEHP on the adult male reproductive system. Here, we aim to investigate how human exposure to DEHP via food consumption may lead to effects on reproduction in adult men. A comprehensive search of the literature was performed to examine human exposures to DEHP, and reported effects of DEHP on the human adult male reproductive system. Five studies were identified that examined occupational exposure to DEHP in men. Four of these studies showed statistically significant relationships between occupational exposures to DEHP and impacts on spermatogenesis. Specifically, occupational exposures to DEHP were associated with decreased sperm concentration, increased sperm motility, increased sperm apoptosis, increased sperm reactive oxygen species (ROS), and effects on various hormones including testosterone, luteinizing hormone, and follicle stimulating hormone. Fourteen studies were identified that involved non-occupational exposure to DEHP, the majority of which demonstrated adverse effects on adult spermatogenesis; the observed effects were similar to those reported in the occupationally-exposed cohorts. However, several of these non-occupational studies also showed no significant effects on spermatogenesis. Although these studies were conducted in non-occupational scenarios, DEHP contributions from food were unclear.

Although there appears to be a correlation between DEHP exposures and altered adult male spermatogenesis, it is unclear how exposure to DEHP via food consumption contribute to the observed effects. Further investigation is needed to determine how these results may be affected by confounding factors and alternate (non-food) exposures to DEHP.

1501 Experimental Rat Cryptorchidism and Susceptibility to Dibutyl Phthalate or Acrylamide

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The increased incidences of male reproductive disorders may be associated with exogenous chemicals such as dibutyl phthalate (DBP) or acrylamide (AA). Cryptorchidism, the most common male congenital abnormality, is variably associated with hypospadias, impairment of spermatogenesis and testicular germ cell tumors. Surgical relocation of the testes to the scrotum, orchiopexy, is performed to prevent these effects. Our hypothesis is that experimental surgical cryptorchidism could enhance testicular susceptibility to exogenous chemicals. Three experiments with Sprague-Dawley rats were performed: 1) in utero and postnatal exposures to DBP or AA until postnatal day (PN) 112; 2) cryptorchidism and orchiopexy established surgically at PN 21 and PN 42, respectively, with termination on PN 98; and 3) in utero and postnatal exposures to DBP or AA, cryptorchidism and orchiopexy established surgically at PN 21 and PN 42, respectively, with termination on PN 112. Pregnant dams, F1 litters and F1 males were exposed to the chemicals by gavage, through milk, and by diet, respectively. The testes were evaluated histologically. The seminal vesicles, epididymis and testes were scored according to the severity of the lesions with the controls being 1.0. 1) Rats exposed only to DBP (score 1.5) or AA (score 1.1) presented a mostly preserved spermatogenesis. Some seminiferous tubules showed vacuolated germinative epithelium, germ cell apoptosis and a Sertoli cell only (SCO) pattern. 2) Cryptorchidism (score 3.3) resulted in decreased absolute testes weights, SCO tubules and spermatogenesis arrest. These alterations were reversed by orchiopexy (score 1.1). 3) Cryptorchidism/orchiopexy associated with continuous exposure to DBP (score 2.5) or AA (score 2.5) resulted in smaller absolute testes weights, spermatogenesis arrest, germ cell exfoliation and multinucleation, and SCO tubules. These data show that surgical cryptorchidism sensitizes the testes to DBP or AA.

1502 Interaction between Mono-(2-Ethylhexyl) Phthalate and All-Trans Retinoic Acid Alters Development of Ex Vivo Cultured Fetal Male Testis

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Toxic effects of phthalate esters (phthalates) on the fetal testis are well-characterized, including reduced testosterone, altered seminiferous cord development, induction of multinucleated germ cells, and germ cell death. The anti-androgenic effects of phthalates vary across species more than seminiferous cord effects, suggesting that additional mechanisms are likely to be involved in phthalate toxicity. We have previously shown evidence that exogenous all-trans retinoic acid (ATRA) reduces seminiferous cord number, alters expression of genes related to sex determination, and induces expression of the ovarian protein FoxL2 in cells of rat fetal testes. Further, simultaneous administration of mono-(2-ethylhexyl) phthalate (MEHP) modulates the effects of ATRA on the rat fetal testes. We hypothesized that disruption of retinoic acid signaling is a mechanism of phthalate toxicity in mice, as well as rats. To test this hypothesis, mouse fetal testes were isolated on gestation day 14, cultured ex vivo with 10−6 to 10−4 M MEHP, 10−5 M ATRA alone, or 10−5 M ATRA plus 10−5 to 10−4 M MEHP. Seminiferous cord development was assessed by quantifying cord number per section in histological sections of cultured testes. FOXL2 expression was assessed by immunohistochemistry. As previously observed in the rat, ATRA exposure caused a significant decrease in seminiferous cord number. Co-exposure with MEHP resulted in a non-linear dose-response indicating an interactive effect of ATRA and MEHP on cord development. Preliminary FOXL2 analysis showed increased FOXL2 staining in ATRA and co-exposure samples. These results provide evidence that MEHP and ATRA interact to disrupt mouse fetal testes development and support the hypothesis that retinoic acid signaling is a target for phthalate toxicity in mice.

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1503 Metabolite Profiling in Mouse Testis Fragments Treated with Endocrine Disrupting Chemicals

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The purpose of this in vitro model of mouse testis organs was to evaluate it as a reproductive toxicity test. In a previous published study, (Sato et al) was first to realize mouse testis fragments cultured in this manner could produce viable, fertilization-proven, sperm, which makes this method a possible alternative form of male reproductive toxicity testing. To evaluate this culture system, we exposed the cultured testis fragments to two known endocrine disrupting chemicals: ethinylestradiol (EE), and dibuthyl phthalate (DBP). Toxicological analysis using liquid chromatography/mass spectrometry (LC/MS)-based metabolomics was used to measure any metabolome changes in the chemical treated testis fragments. Testes from postnatal day 5, C57BL/6J mice were collected, testicular tunica removed, and the testes cut into four similar sized fragments. Two to three testis fragments were placed onto a 1.5% agarose gel cube and cultured in α-MEM including 0.4% Albumax I (day 0). On day 1 of culture, testis fragments were treated with 0.01 or 1 nM EE; or 0.1 or 10 μM DBP or vehicle (control). On day 20 of culture, the testis fragments were collected for LC/MS and histology analysis. Histological analysis demonstrated the percentage of seminiferous tubules with any dead cells increased dose-dependently. Metabolic analysis demonstrated metabolites involved in glycolysis pathway (UDP-glucose, glucose phosphate and pyruvate), in tricarboxylic acid cycle pathway (oxaloacetate, citric acid and aspartate), and arginine-creatine metabolism pathway (creatine, arginine, spermine and spermidine) and their altered levels by both EE and DBP treatments. These findings suggest that EE and DBP treatments might cause testicular toxicity with similar underlying mechanisms since both chemicals disturbed similar metabolism pathways but with varying results. To confirm these findings, further experiments using other testicular toxicants will be necessary.

1505 Gestational ENM Inhalation Compromises Placental Efficiency


Maternal engineered nanomaterial (ENM) inhalation during gestation is associated with uterine vascular impairments and endocrine disruption that may lead to altered gestational outcomes. We have previously shown that nanotitanium dioxide (nano-TiO2) inhalation impairs endothelium-dependent arteriolar dilation in uterine arteries of pregnant rats, and significantly decreases plasma estrogen. However, the mechanism underlying this dysfunction is not fully described. Fetal death has been shown in vivo and in vitro for synthesis of the hormone kisspeptin (Kiss), which is a potent vasoonconstrictor. Therefore, we examined how Kiss is involved in nano-TiO2-induced vascular dysfunction and placental efficiency. Pregnant (gestational day (GD) 6) Sprague-Dawley rats were exposed to nano-TiO2 aerosols (31.1 ± 1.1 μg per day; cumulative dose = 29.7 ± 1.0 μg; n = 6) or sham exposed (n = 6) to evaluate consequences of maternal exposure. Rats were sacrificed 24 hours after last exposure, at GD 20. Plasma was collected to evaluate circulating Kiss level. Wet and dry weights of placents and pups were measured and used to calculate placental efficiency (grams fetus/gram placental). Additionally, pressure myography was used to determine vascular reactivity in uterine arteries (nano-TiO2 exposed n = 7; sham n = 6). Uterine arteries were equilibrated for 1 h prior to experimentation. Contractile responses were determined via cumulative additions of 50 μL of Kiss (1 × 10⁻⁴ to 1 × 10⁻⁴ M). The steady-state diameter of vessels was recorded for at least 2 min after each dose. No difference was seen in plasma Kiss between groups. Wet placental weights were significantly increased in exposed (0.99 ± 0.03 g) versus sham rats (0.70 ± 0.04 g), whereas wet pup weights were significantly decreased in exposed rats (4.01 ± 0.47 g vs. 4.15 ± 0.15 g). These alterations led to a significant decrease in placental efficiency in exposed rats (4.52 ± 0.20) compared to sham (6.35 ± 0.5). Maternal ENM inhalation exposure significantly increased constriction (91.2% ± 2 vs. 98.6% ± 0.1) in uterine arteries when treated with Kiss. These studies represent evidence that pulmonary ENM exposure perturbs the normal gestational endocrine vascular axis via a Kiss dependent mechanism. Additionally, a decrease in placental efficiency due to exposure is likely to have long term consequences on adult health and morbidity. Support: ES015022 (TRN); U54GM104942 (Sh).

1504 Reproductive Toxicity and Oxidative Damage Induced by Titanium Dioxide, Zinc Oxide Nanoparticles, and Their Mixture in Mice

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The continuous release of Titanium dioxide (TiO₂) and Zinc oxide (ZnO) nanoparticles (NPs) from consumer products into the environment warrants safety risk assessment, as they may induce reproductive anomalies. There is dearth of information on interactive effects of TiO₂, ZnO NPs and their mixture (1:1) to alter spermatogenesis, reproductive hormone and oxidative stress parameters. The shape, hydrodynamic diameter (HD), and zeta potential (ζ) of TiO₂ and ZnO NPs were characterised using the transmission electron microscopy and dynamic light scattering, respectively. Swiss albino mice were intraperitoneally administered 9.38 - 150.00 mg/kg bw each of TiO₂ (100 nm) and dynamic light scattering, respectively. Swiss albino mice were intraperitoneally administered 9.38 - 150.00 mg/kg bw each of TiO₂, ZnO NPs and their mixture (1:1) and the mixture treated testis fragments. Testes from postnatal day 5, C57BL/6J mice were collected, testicular tunica removed, and the testes cut into four similar sized fragments. Two to three testis fragments were placed onto a 1.5% agarose gel cube and cultured in α-MEM including 0.4% Albumax I (day 0). On day 1 of culture, testis fragments were treated with 0.01 or 1 nM EE; or 0.1 or 10 μM DBP or vehicle (control). On day 20 of culture, the testis fragments were collected for LC/MS and histology analysis. Histological analysis demonstrated the percentage of seminiferous tubules with any dead cells increased dose-dependently. Metabolic analysis demonstrated metabolites involved in glycolysis pathway (UDP-glucose, glucose phosphate and pyruvate), in tricarboxylic acid cycle pathway (oxaloacetate, citric acid and aspartate), and arginine-creatine metabolism pathway (creatine, arginine, spermine and spermidine) and their altered levels by both EE and DBP treatments. These findings suggest that EE and DBP treatments might cause testicular toxicity with similar underlying mechanisms since both chemicals disturbed similar metabolism pathways but with varying results. To confirm these findings, further experiments using other testicular toxicants will be necessary.

1506 Dual Hit—Uteroplacental Hypoxia after Engineered Nanomaterial Exposure

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Uteroplacental vascular remodeling and hemodynamic control are central to delivering oxygen and nutrients to support the placenta and developing fetus during pregnancy. Hypoxia has been identified as a contributing factor to placental dysregulation and the development of maternal-fetal disease, including intrauterine growth restriction (IUGR). Our laboratory has demonstrated that rodent exposure to engineered nanomaterials (ENM) early in pregnancy (GD 4) causes IUGR as evidenced by smaller placentas, more fetal reabsorption sites, and reduced fetal pup number and size. However, the cellular and molecular mechanisms that culminate to IUGR remain elusive. Therefore, we initiated a set of experiments to assess hypoxic signaling and placental adaption to direct ENM exposure. Using in vitro methodology, BeWo b30 trophoblast carcinoma cells were exposed to increasing concentrations of 20 nm TiO₂ (1, 10, 100 μg/mL) and analyzed with qPCR for changes in hypoxia signaling markers after 24 hours. At 100 μg/mL HIF1α (2.24 ± .31), eNOS (1.95 ± .27), VEGF-A (2.25 ± 3.3) and VEGFRI (2.03 ± .67) mRNAs were up-regulated. These markers were also elevated in western blot analyses. Exposures of human term placental explants presented similar findings, wherein HIF1α (2.1 ± .60), eNOS (1.3 ± .43), and VEGF-A (1.26 ± .72) mRNAs were upregulated. In ex vivo studies using rodent placental perfusion methodology, infusion of 900 μL of 20 nm rhodamine-labeled polyisoyrene or 20 nm gold sonicated in PSS into the maternal uterine artery significantly reduced fluid flow through the maternal uterine artery compared to baseline (after 60 minutes by 40% and 90 minutes by 55%), respectively. Moreover, fluid flow was significantly reduced through the fetal umbilical vein into fetal compartment as compared to baseline (within 10 minutes by 40% and 70 minutes by 60%, respectively). Overall, these studies indicate the upregulation of placental hypoxia signaling markers after direct ENM-trophoblast exposure. Further development of a hypoxic environment is enhanced given the decrease in fluid flow across the placenta after ENM infusion. These results, in combination with published work demonstrating uterine microvascular constriction after maternal ENM exposure demonstrate the development of a hypoxic uterine environment after ENM exposure. NIH-R00-ES024783 (PAS); T32-ES070148 (JND, JTS, LMA); P30-ES000022.
Numerous epidemiological and animal studies have demonstrated that exposure to ambient fine particulate matter (<2.5 μm in diameter [PM$_{2.5}$]) during gestation is associated with adverse obstetric outcomes including preterm birth (PTB). Early delivery has been linked to several lifetime health consequences for offspring, including behavioral and psychological abnormalities, and reduced immune and respiratory functions. In a previous study performed in this laboratory, B6C3F1 pregnant mice exposed to concentrated ambient PM (CAPs) by inhalation, demonstrated shortened (by 0.4 d) gestational duration compared to filtered air (FA) controls. The mechanisms underlying the association between PM$_{2.5}$ and PTB are not currently well understood. Since the placenta provides a crucial link between the intrauterine environment and fetal growth/development, it is a major target of PM and key for studying the effects on birth outcomes. Therefore, in this study, placentae from the previously developed pregnant mouse model (n=6 each from CAPs and FA groups) were subjected to whole transcriptomic profiling by RNAseq. A bioinformatic RNAseq analysis workflow (trimport, Salmon and edgeR) was used to identify differentially expressed genes between treatment groups. The 648 genes from the curated dBPTB (database Preterm Birth) were used for a candidate gene approach and were examined using gene counts obtained from RNAseq. Following PM$_{2.5}$ exposure, six PTB genes were downregulated in placentae (Ace, Ddah1, Col1a2, Chst15, Akap12, Ephx1) and one was upregulated (Chys3) (p<0.01). Gene Ontology demonstrated that these seven genes are involved in neutrophil-mediated immunity, arterial blood pressure regulation, amino acid binding, cell membrane function, metal ion binding, and aromatic compound catabolism among other functions that could be linked to PTB. Additional computational models, agnostically testing placental transcriptome-wide differential gene expression, revealed additional genes that were differentially expressed between the two treatment groups. These findings suggest that PM exposure influences the placenta genome thereby mediating PTB. Identification of these differentially expressed genes may contribute to intervention strategies to mitigate these adverse effects. Supported by: March of Dimes, NIEHS P30ES000206, and NIEHS P30ES0232315.

In the United States, the number of female welders has increased by more than two percent over the past decade. The U.S. Bureau of Labor Statistics predicts steady industry growth through 2024, and it is expected that many women will continue to fill these roles as the baby-boomer generation prepares to retire. Notable data currently exists on the potential reproductive effects of mild and stainless steel welding fumes on the placenta. Using human placental trophoblast cells (HTR-8/SVneo) from the first trimester, we aimed to identify the mechanisms of toxicity associated with stainless steel (SS) and mild steel (MS) welding rods. MS welding fumes are mainly composed of iron and manganese, while SS-welding fumes primarily contain hexavalent chromium and nickel. During embryogenesis and placentaion, cellular migration is a highly orchestrated and multi-step process that plays an integral role in providing the foundation for a successful pregnancy. In this study, exposure of HTR-8/SVneo cells to 100 μg/mL of SS welding fumes for 24 h using the RadiusTM Migration assay showed significant inhibition of cellular migratory ability, whereas cells exposed to MS were not affected. Using electron paramagnetic resonance, exposure of cells to SS also produced greater amounts of the hydroxyl radical when compared to MS. Results from a multiplex cytokine kit (Meso Scale Diagnostics, LLC) show that exposure of cells to SS causes a pro-inflammatory response, with significant increases of IL-8 and IL-15 observed. Finally, scanning electron microscopy was performed to better understand how particles are internalized by placental cells. For both MS and SS, welding fumes accumulated in vascular spaces, which could potentially explain the increased Endothelin-1 results observed using ELISA. Endothelin-1 is a potent vasoconstrictor that is necessary for fetal formation, but upregulated in the setting of inflammation. Our data showed that SS appears to have the most damaging effect on placental cells, which could be due the presence of hexavalent chromium, not found in MS. Further studies are needed to delineate the toxicity of the individual metals found in welding fumes and their effects on the female reproductive system.

Ovarian follicles progress from a primordial stage through primary, secondary, antral and finally preovulatory stages. The finite pool of primordial follicles constitutes the total ovarian follicle reserve. Exposure of female rodents to polycyclic aromatic hydrocarbons (PAHs) results in destruction of immature primordial and primary follicles. PM$_{2.5}$ is rich in PAHs, which are adsorbed onto particle surfaces; the effects of exposure to PM$_{2.5}$ on the ovarian reserve have not been investigated. Apolipoprotein (Apo) E null mice were exposed to concentrated ambient PM$_{2.5}$ or filtered air for 12 weeks, 5 days per week for 4h/day using a Versatile Aerosol Concentration Enrichment System. ApoE null mice are predisposed to develop atherosclerotic plaques; these mice were part of a study investigating vascular toxicity of PM$_{2.5}$. Mice were euthanized on the day of proestrus of the estrous cycle 24h after the final exposure. One ovary per mouse was processed for follicle counts. Primordial and primary follicles were counted blind to treatment in every 4th 20 μm section on a stereology system using the optical fractionator method. Secondary and antral follicles were followed through every section. Primary follicle numbers were significantly decreased by 51% (P=0.007, t-test), and primordial follicle numbers were nonsignificantly decreased (P=0.13) in PM$_{2.5}$-exposed mice. The overall small follicle count (primordial plus primary) was significantly decreased by 45% (P=0.03). Numbers of healthy secondary follicles that eventually become antral and cortical stage follicles were reduced by 22% in PM$_{2.5}$-exposed mice. The overall small follicle reserve was decreased by half in PM$_{2.5}$-exposed mice. Decreased ovarian reserve leads to premature ovarian failure, which increases the risk for cardiovascular disease in women. Thus our data provide a potential link between ovarian and cardiovascular effects of exposure to PM$_{2.5}$. Ongoing work is investigating the mechanism of follicle depletion. Supported by California Air Resources Board, 16DR005.

Chrysotherein (TCE), is an industrial solvent and widespread Superfund site contaminant. Despite epidemiological evidence associating exposure with adverse birth outcomes, the effects of TCE and its metabolite S-(1, 2-dichlorovinyl)-L-cysteine (DCVC) on the placenta remain undetermined. Theaproposed exposure and utilization of biofuels and production of small molecules is rich in metabolic and mitochondrial function are essential for placental cells. Our study investigated the effects of DCVC exposure on energy metabolism and mitochondrial function in placental cells. Human extravillous trophoblast cells, HTR-8/SVneo, were exposed to 5-20 μM DCVC for 6 or 12 hours. Targeted metabolomics were used to evaluate small molecule and metabolite concentrations from different energy metabolism pathways. Our experiments demonstrated an early increase in glycolysis as well as a partial upstream obstruction in the glycolytic pathway, resulting in lipid breakdown as a source of biofuel to provide intermediate glycolytic substrates that enter the pathway downstream of the obstruction. Additionally, an initial increase in TCA cycle activity was observed followed by a slight decrease in activity. Using a Seahorse XF Analyzer for monitoring concurrent oxygen consumption rate and extracellular acidification rate in real time, our experiments also demonstrated an increase in basal oxygen consumption, mitochondrial proton leak and decrease in energy coupling efficiency accompanied by sustained extracellular acidification indicative of glycolytic activity. These changes were followed by observed decreases in mitochondrial-dependent basal and maximum oxygen consumption rates and dissipation of mitochondrial membrane potential. Taken together, these results suggest that DCVC exposure causes substantial energy stress, necessitating alterations in biofuel sources and energy metabolism pathway utilization, further complicated by progressive mitochondrial dysfunction and resulting in decreased physiological adaptability. Our findings demonstrate the biological plausibility of DCVC-induced placental toxicity and provide new insights into the toxicological mechanisms of action of TCE and its metabolite DCVC.
Trichloroethylene (TCE) is an environmental contaminant shown to be associated with low birth weight and small for gestational age in human epidemiological studies, decreased fetal weight in Wistar rats, and impaired mitochondrial function in placental cells. Building upon this information, the present study conducted tricarboxylic acid (TCA) Plus and short chain fatty acid (SCFA) targeted metabolomics analysis on amniotic fluid samples to identify changes in metabolites induced by exposure to TCE. Rats were treated with 480 mg TCE/kg/day from gestational day (GD) 6 to GD 16, and amniotic fluid samples were collected on GD 16. We observed a statistically significant decrease for both fetal genders for the following metabolites: 6-phosphogluconate (6PG), guanosine diphosphate (GDP), adenosine diphosphate (ADP), adenosine triphosphate (ATP), and flavin adenine dinucleotide (FAD). Some metabolites decreased in a gender-specific manner including the following: fructose 1,6-bisphosphate (FBP) (male only), guanosine triphosphate (GTP) (male only), acetyl-CoA (aCoA) (male only), and uridine diphosphate (UDP)-D-glucuronate (female only). Finally, several lipid metabolites were both increased with TCE treatment for females only. Together, these results highlight alterations in indirect or direct energy supply (6PG, GDP, ADP, ATP, FAD, FBP, GTP, aCoA, phosphocreatine) and oxidative stress content (arginine, UDP-D-glucuronate) in the amniotic fluid as part of the response to TCE exposure. This also indicates that TCE exerts different effects on amniotic fluid metabolites in male and female fetuses. Future research is warranted to fully understand how these alterations could be part of the mechanism by which TCE exerts its toxicity during pregnancy.

Etiological, Clinical and Data Base Analyses of Human Infertility in USA, Suggest the Role of Environmental Pollutants in Increasing Infertility for Industrial and Urban Populations

Infertility in humans has been considered to be one of the impending health issues of social and economic aspects. As a whole, infertility is a very complex health issue involving multiple etiological aspects, such as reproductive, endometrial, environmental and genetic factors. There are several reports that warn about the progressively diminishing fertility rates around the globe. Both male and female factors have been attributed for the reduced fertility rates. Infertility rate is higher in USA than most of the industrial countries. There are no studies to investigate the factors involved in infertility rates from various states of USA. Hence, a comprehensive data base analysis (CDC data base, population data base, PubMed entrez and North Carolina State data bases) for the following aspects were undertaken: A) Fertility rate based on the birth rate information of various states, B) Fertility rate of individual countries in North Carolina as model state, and C) Collecting etiological data relevant to reproductive aging, important developmental periods, infectious diseases, chronic conditions and diseases, behavioral factors, iatrogenic causes, occupational and environmental hazards, and genetic influences. Based on the data analyses the following conclusions may be derived: A) Industrialized states tend to have lower fertility rate, B) Exposure to heavy metals (such as lead, zinc, mercury, cadmium, nickel) pollution may be one of the causes, and C) Differential distribution of the above mentioned etiological factors may have synergistic outcome on the fertility of the couples. Furthermore, the male and female related infertility factors may have differential distribution as per socio economic status, rural vs. urban life style and other unknown factors.

NTP Monograph on the Systematic Review of Occupational Exposure to Cancer Chemotherapy Agents and Adverse Health Outcomes

Many cancer chemotherapy agents are developmental toxicants, genetic toxicants, and/or known carcinogens. Despite the issuance of safe handling guidelines in 1980s, occupational exposure continues to occur. Based on the evidence of carcinogenicity and genetic toxicity associated with direct administration of these agents and current evidence of occupational exposure, NTP conducted a systematic review to: 1) evaluate whether occupational exposure is associated with any adverse health outcomes in humans, and 2) summarize the prevalence and levels of chemotherapy agents in the workplace measured by environmental monitoring and biomonitoring. A literature search was performed up to February 23, 2017 using PubMed, Embase, Scopus, Toxline, and Web of Science. Data were extracted, and risk of bias was assessed from relevant studies. Studies were grouped on an outcome basis, and these bodies of evidence were assessed to develop confidence ratings and level-of-evidence conclusions. The literature search and screening identified 110 epidemiological studies assessing possible adverse health outcomes and 171 studies evaluating exposure. NTP concluded that there is a moderate level of evidence that occupational exposure to cancer chemotherapy agents is associated with a higher incidence of spontaneous abortion, particularly when evaluating studies of nursing and pharmacy personnel. NTP also concluded that there is a moderate level of evidence that exposure to these agents in the workplace is associated with genetic toxicity based on consistent reports of higher levels of structural chromosome aberrations, micronucleus induction, and DNA damage measured by comet assay. Due to few studies per outcome and heterogeneity in the data, there was inadequate evidence to reach level-of-evidence conclusions on other health outcomes. Cancer chemotherapy agents were commonly detected in environmental samples of the workplace and urine or blood of workers handling these agents, including in data collected as recently as 2014 to 2016. Considering the potential for occupational exposure to these agents and their association with spontanous and reproductive effects during therapy, it is necessary to reduce exposures through training in safe handling procedures and provision and use of personal protective equipment and associated safety containment equipment. Identified research needs and data gaps included improved exposure characterization and broadening the range of workers monitored for exposure.
## 1515 Development of a Liquid Chromatography-Mass Spectrometry Method to Quantitate Deoxynivalenol in Harlan Sprague-Dawley (HSD) Rat Plasma, Amniotic Fluid and Fetal Homogenate


Deoxynivalenol (vomitoxin or DON) is a mycotoxin primarily produced by fungi of the Fusarium genus. It may be found in Fusarium-infected wheat, corn, and barley and hence there is potential for human exposure. Although some toxicity data exists for DON, data following perinatal exposure is generally lacking. The objective of this work was to develop a method to quantify DON in HSD rats to assess fetal and lactational transfer of DON in support of a National Toxicology Program (NTP) toxicology study. Standards were prepared by spiking 100 μL of matrix with 20 μL of DON spiking solution and 20 μL of 13C10-DON (internal standard, IS) in acetonitrile. Samples were extracted by adding 360 μL of acetonitrile followed by vortex mixing and centrifugation. The supernatants were dried, the residue was reconstituted in 150 μL of 20% aqueous acetonitrile and analyzed by liquid chromatography-tandem mass spectrometry. An Acquity UPLC BEH C18 column was used with mobile phases consisting of water and methanol. The electrospray ionization source was operated in negative ion mode. The MRM transitions were 295/265 and 310/279, for DON and 13C10-DON, respectively. The method was successfully qualified over a calibration range ~2 to 1000 ng/mL in male HSD rat plasma. The matrix standard curve was linear (r > 0.99). The lower limit of quantitation was 2.06 ng/mL and the limit of detection (LOD) was 0.354 ng/mL. The accuracy (determined as % relative error, RE) and precision (determined as % relative standard deviation, RSD) for the lowest matrix spiked level was ≤± 12% and 5.5%, respectively. The method was qualified for GD18 HSD dam plasma and fetal homogenate (in water) at ~5 and 500 ng/mL DON was stable in HSD GD18 maternal rat plasma and fetal homogenate when stored at ~70 °C for at least 29 and 43 days, respectively. The method was applied to samples from a dose range finding study where HSD dams were dosed via gavage from GD 6 to PND 28 with either 0 (control) or 1 mg/kg/day DON. DON was quantified in GD 18 dam plasma (17.7 to 21.1 ng/mL), amniotic fluid (9.65 to 14.8 ng/mL), and fetal homogenate (21.5 to 24.7 ng/mL) demonstrating fetal transfer. Although DON was found in PND 4 dam plasma (16.2 to 18.6 ng/mL), the levels in pup plasma were below the LOD suggesting absence of transplacental transfer.

## 1516 Propanol Acutely Changes Prolactin, Estrogen and Splenic Cell Populations in Female Mice


The herbicide propanol is used in rice fields globally. Earlier research has indicated that propanol enhances adaptive immune responses to heat-killed Streptococcus pneumoniae (HKS) immunization. This enhancement is dependent upon an intact hypothalamic-pituitary-gonadal (HPG) axis in female mice. It has been hypothesized that propanol alters reproductive hormones (progestosterone, prolactin and estradiol) as well as splenic immune cell populations as early as 24 hours in HKS-immune responses, in a gonadotropin releasing hormone (GnRH) receptor-dependent manner. To test this, 48 C57BL/6 female mice were assigned among 8 groups (6 mice each) in a 2x2x2 factorial arrangement of treatments: propanol (200 mg/kg i.p.), HKS (2x10^6 cfu i.v.) and GnRH receptor antagonist (antide; 60 ug i.q.) and their respective controls. Serum, spleens, ovaries and uteri were harvested 24 hours post immunization. Progesterone, prolactin and estradiol concentrations were measured by specific ELISA. Splenic T cells (CD4+ and CD8+), B cells and CD11b+ cells were analyzed by flow cytometry. Propanol increased the concentrations of prolactin (p=0.006) and the percentage of CD4+ cells (p=0.037) in the spleen, and it decreased concentrations of serum estradiol (p=0.0001), ovarian (p=0.022) and spleen (p=0.001) weight, correspondingly to decrease in numbers of splenocytes (p=0.028), B cells (p=0.017), and CD4+ cells (p=0.043) when compared to mice without propanol treatment. Propanol did not alter progesterone concentrations. Antide as a main effect reduced uterine (p=0.035) and ovarian (p=0.0001) weight, and ovarian area (p=0.0006). The percentage of CD11b+ cells (p=0.008) and CD4+ cells (p=0.006) in the spleen were also reduced, while spleen weight (p=0.006), numbers of splenocytes (p=0.046), B cells (p=0.017), and percentage of B cells (p=0.012) were increased. However, antide did not have interactive effects with propanol on any responses. In summary, antagonizing GnRH receptors with antide affected immune and reproductive variables but it did not modify propanol’s acute effect. This suggests the GnRH receptors are not necessary in the acute phase (24 hrs) of propanol/HKS challenge but that does not exclude other potential components of HPG axis.

## 1517 Disruption of Cholesterol Homeostasis through the Retinoid X Receptor in Human Ovarian Granulosa and Theca Cells after a Low-Dose Tributyltin Exposure

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Endocrine disrupting chemicals (EDCs) have been shown to interfere with reproductive function leading to sub- or infertility. Tributyltin (TBT) is one of such EDCs used as a chemical catalyst and biocide with human exposure ranging between ~0.1 and 85 ng/mL. We have demonstrated that TBT can stimulate cholesterol efflux in ovarian theca cells of four mammalian species. Since cholesterol is the steroid hormone precursor in steroidogenic cells, we aimed to investigate if a short-term, low dose TBT exposure will disrupt cholesterol homeostasis in human primary ovarian steroidogenic cells (granulosa and theca cells). To test this hypothesis we obtained human ovaries from 6 subjects (31–48 years old, without prior tumorigenesis or hyperandrogenism) from Sparrow Hospital (IRB: 17-1066M). Granulosa and theca cells were isolated and purified (n=3 for each cell type) with collagenase and cell density gradient. Cell viability and cytotoxicity were assessed by trypan blue stain and MTT assay, respectively. We tested pre-luteinized cells and luteinized (in vitro luteinization induction with LH) cells. Human granulosa and theca cells were exposed to an environmentally relevant dose of TBT (10ng/mL) and/or retinoid X receptor (RXR) antagonist (UV3003; 8μM) for 48h (pre-luteinized cells) or 72h (during luteinization). Steroidogenic enzymes and cholesterol transport genes were measured by RT-qPCR. Pre-luteinized and luteinizing granulosa and theca cells exposed to TBT upregulated ABCA1 expression. GREB1 upregulation was observed in pre-luteinized granulosa and theca cells. STAR was only upregulated in pre-luteinized granulosa cells, which may be a compensatory effect from TBT-induced ABCA1 upregulation. HSD3B1 and 17BHS expression remained unchanged. The RXR antagonist blocked the TBT-induced ABCA1 upregulation in both cell types. In conclusion, TBT, at an environmentally relevant dose, stimulates cholesterol extracellular efflux via the RXR pathway. Along with our work in other mammalian species, these results are supportive of TBT’s conserved mechanism on cholesterol trafficking in steroidogenic cells. The low dose used falls within current human exposure levels and thus warrants further work to understand the impact of organo-tin-induced cholesterol disruption in steroidogenic cells on female fertility. Supported by NIAMS 1K22ES026208 to AV-L.

## 1518 Sirtuin-1 Inhibitor EX-527 Hyperacetylates p53 and Attenuates CrVI-Induced Germ Cell Apoptosis


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Environmental contamination with hexavalent chromium (CrVI) has been increasing in the United States as well as in the developing countries. Due to increased use of Cr and improper disposal of Cr wastes from the industries, CrVI levels in the soil, water and air have been constantly increasing. Exposure to CrVI predisposes human population to various diseases including cancer, infertility, and developmental problems in children. Previous findings from our laboratory reported that prenatal exposure to CrVI caused premature ovarian failure through p53-mediated mechanisms. Sirtuin 1 (SIRT1) is a NAD+-dependent histone deacetylase (HDAC) class III. SIRT1 deacetylates several histone and non-histone proteins such as p53 and NFκB. Current study determines a role for SIRT1-p53 network in CrVI-induced germ cell apoptosis and establishes physical interaction between SIRT1 and p53. Adult pregnant dams were given regular drinking water or CrVI (10 ppm potassium dichromate in drinking water, ad libitum), and treated with three different doses of SIRT1 inhibitor EX-527 (1, 5 and 50 mg/kg body weight, i.p.), during 9.5 -14.5 days post-coitum. On postnatal day-1, ovaries from F1 offspring were collected for various analyses. Results showed that CrVI increased germ cell apoptosis, down regulated SIRT1, upregulated acetyl-p53, activated apoptotic pathway, and inhibited cell survival pathway. CrVI inhibited physical interaction between SIRT1 and p53 in a spontaneously immortalized rat granulosa cell line. Treatment with SIRT1 inhibitor attenuated CrVI-induced mechanisms in a dose-dependent manner. Taken together, the present study suggests that targeting SIRT1 may be an ideal approach to rescue ovaries from CrVI-induced apoptosis.
**1519 Ataxia Telangiectasia Mutated Coordinates the Response to Phosphoramide Mustard-Induced Ovarian DNA Damage**

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Phosphoramide mustard (PM), an ovotoxic metabolite of cyclophosphamide (CPA), is used in the treatment of autoimmune diseases and in anti-cancer chemotherapy, as it destroys rapidly dividing cells by inducing DNA double strand breaks (DSB). The ataxia telangiectasia mutated (ATM) protein is critical in the recognition and repair of DNA DSBs through activation of cell cycle checkpoints and DNA repair proteins. ATM gene mutations increase cancer risk, particularly breast and ovarian cancer. We and others have demonstrated that PM exposure leads to ovarian DNA damage and destruction of ovarian follicles, resulting in premature ovarian failure. Additionally, we have discovered that ATM inhibition prevents PM-induced follicular depletion, though the effects on oocyte quality remained to be determined. Thus, we hypothesized that the DNA damage response would be blunted in Atm−/− deficient mice and unhealthy follicles that normally would be triggered for repair and/or apoptosis would remain, resulting in impaired fertility and increased amounts of retained DNA damage. Briefly, wild-type (WT) C57BL/6 or Atm−/− were intraperitoneally dosed once with sesame oil (95%) or PM (25 mg/kg) and ovaries harvested 3 days thereafter. Body weight was recorded pre-dosing and vaginal cytology was monitored. The WT mice were heavier (P = 0.016) than the Atm−/− counterparts. Additionally, relative to WT controls, Atm−/− mice spent more time in the diestrous phase of their estrous cycle (P = 0.0009), indicative of premature ovarian aging. PM exposure induced (P < 0.05) loss of primordial, primary, secondary, and antral follicles in WT mice, however, in the Atm−/− mice, there was a depletion of primordial and primary but no impact secondary or antral follicle number. Interestingly, relative to the effects of PM exposure in WT mice, the reduction in primordial follicle number was lessened with ATM deficiency. Immunofluorescence staining revealed that PM induced the DNA damage marker, γH2AX, in the oocyte and granulosa cells, but that this response was diminished in follicles isolated from Atm−/− mice relative to the WT controls. In addition, there was a reduction in the level of cleaved caspase 3 in the Atm−/− PM-treated mice, relative to the WT counterparts. These data support roles for ATM in ovarian DNA repair and atresia and indicate the importance of this pathway in maintaining oocyte quality. Supported by the Bailey-Career Development Award from Iowa State University.

**1520 Estrogenic Activity of Polycyclic Aromatic Hydrocarbon Metabolites in Human Endometrium**


Polycyclic aromatic hydrocarbons (PAHs) are 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 uterine toxicants in rodents. Moreover, PAHs or their metabolites can activate estrogen receptors (ER), resulting in endocrine disruption. Once in the body, PAHs are metabolized by the family of CYP-450 enzymes to more water-soluble hydroxy-PAHs (OH-PAHs). Additionally, alkoxyreductase enzymes (AKRs) convert PAH trans-dihydriodiolis into PAH ortho (o)-quinones. Given the similarity between planar PAH and estrogens, we hypothesize that PAH and PAH metabolites activate ERs in estrogen target tissues e.g. endometrium. We used inducible alkaline phosphatase activity in Ishikawa cells, a human endometrial adenocarcinoma cell-line, and estrogen response element (ERE) luciferase activity as the read-out for ER activation. We tested the estrogenicity of various PAH and their metabolites, including benzo[a]pyrene (BaP), 3 PAH o-quinones (benzo [a] pyrene-7,8-dione (BPQ), benzo[a]anthracene-3,4-oxide and 3-methylcholanthrene-1,2-dione), and 3- OH-BaP in endometrial cells. We demonstrated that these compounds can induce ER activity with EC50 values in the low micromolar range, and that this activity is inhibited by Fulvestrant, an ER antagonist. We have also shown that nanomolar concentrations of BPQ induce the translocation of ERα into the nucleus to modulate cell cycle gene expression. Using high performance liquid chromatography and APC1 mass spectrometry in the selected reaction monitoring mode, we find that BaP can be metabolized to the estrogenic BPQ and 3-OH-BaP in Ishikawa cells in sufficient amounts to activate ER. Low nanomolar concentrations of BPQ increase Ishikawa cell proliferation to the same level observed with picomolar concentrations of 17-β-ethinyl estradiol, and both effects are blocked with Fulvestrant. Our work indicates that planar PAH metabolites may play a role in the disruption of ER signaling in the endometrium. Additionally, differences in reporter gene response and cell proliferation were noted, raising the question of whether or not the estrogenic effects are mediated through ER.

**1521 Chromium VI Exposure During Pregnancy Disorganizes Uterine Tissue Remodeling**

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Hexavalent chromium (CrVI) is a heavy metal environmental endocrine disruptor. CrVI has been shown to be associated with a wide range of applications in the chemical industry, graphics industry, artistic paints, anticorrosion paints, electropolating, steel alloys, stainless steel welding, and a multitude of other uses. Increased use and improper disposal of Cr in the developing countries and in the US have increased Cr levels in the air, soil and drinking water. Significant contamination with CrVI has been found in approximately 30% of the drinking water sources in California. For a California chromium production group, Cr has been found in high levels in the drinking water sources from more than 34 cities in the US Women working in Cr industries and living in Cr contaminated area experience infertility problems and preterm abortions, and still birth. CrVI is rapidly transported inside the cell by specific transport mechanisms, and it leads to deregulate cellular differentiation 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 or fetus, genetic influences, behavioral and socioeconomic factors and iatrogenic prematurity. Maternal exposure to environmental contaminations has been considered as an important contributing factor. CrVI crosses the placenta. We hypothesize that pre-natal exposure to chromium disorganized uterine tissue remodeling by altering collagens, elastin, MMPs and TIMPs, as well as Xnpep2 that hydrolyses collagen. Pregnant rats (n=10) were treated or not treated with 5 ppm CrVI (potassium dichromate 5 mg/liter drinking water) from gestational days (GD) 0.5 to 4.5. On GD 18.5, the dams were euthanized by CO2 asphyxiation followed by cervical dislocation and the uterine arteries (UA) were collected for further analyses. Results indicated that CrVI reduced fetal weight, induced premature labor, increased uterine arteriolar stiffness, inner wall thickness and reduced the lumen diameter of the UA. Further, CrVI increased the deposition of collagens 1, 3 and 4, and elastin; decreased Xnpep2 and MMPs, thus increasing the UA wall thickness and reducing the lumen diameter of the UA. Thus, our results indicated that CrVI disorganized UA wall thickness, which may lead to restricted blood flow and increased pressure in the UA, leading to the intrauterine growth restriction (IUGR). This study was supported by a grant from NIH/NIEHS to S.K.B (R01ES025234-01A1).

**1522 Adverse Effects of Naphthenic Acids on Reproductive Health: A Focus on Placental Trophoblast Cells**

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The Alberta oil sands have the third largest oil reserves in the world containing an estimated 2.5 trillion barrels of recoverable bitumen. Bitumen extraction generates residual tailings water known as oil sands process-affected water (OSPW). Oil sands process-affected water contains several major classes of contaminants, the toxicity of OSPW has been primarily attributed to naphthenic acids (NA). It has been well established that NAs can negatively impact reproduction in non-mammalian vertebrates, but the effects on mammalian reproduction have largely been overlooked. A recent study reported that treatment of rats with NAs caused a decrease in litter size and birthweight, yet the mechanisms are unknown. As both of these pregnancy outcomes are related to placental dysfunction, the goal of this study was to determine the effects of NA exposure on placental cell function. Methods: Htr8/SVneo cells, a model of first trimester trophoblast cells, were exposed to vehicle, 0.25, 1.25, 25 and 125 mg/L of a commercial technical NA mixture (SigmaAldrich) for 24h; these concentrations are within the range of NA concentrations reported in OSPW. We assessed steroid production (progesterone [P4], estradiol [E2], testosterone [T]) and markers of inflammation ( interleukin-1β [IL1β], prostaglandin E2 [PGE2]) and oxidative stress (heme-oxigenase-1 [HMOX]1) induced in response to oxidative stress. Results: NA treatment significantly altered steroid production; P4 was decreased at all doses tested, whereas there was a significant increase in T production (125mg/L only) and no effect on E2 production. In addition, NA treatment resulted in increased markers of inflammation and oxidative stress. PGE output and expression of PTGS2, the key enzyme in prostaglandin production, was up-regulated in NA treated cells but not in NA treated cells. Not all NAs were PAHs but NA treatment was only seen at the highest dose tested. Similarly, 125mg/L NA significantly increased expression of IL1β and HMOX. Although it is well established that the commercial NA mixtures do not wholly represent those found in OSPW, these results provide proof of concept that NA exposure has the potential to perturb placental cell function. Future work will explore altered steroid production, oxidative stress and inflammatory stress are associated with numerous adverse pregnancy outcomes, these findings suggest that it is biologically plausible that reproductive toxicity of NAs in mammals may be mediated via placental dysfunction.
Inhibition of Cyclic Nucleotide Efflux by Placental MRP Transporters Enhances Trophoblast Syncytialization

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In the placenta, multinucleated syncytiotrophoblasts arise from the fusion of cytotrophoblasts, a process regulated by cyclic nucleotide (cAMP and cGMP) signaling. Syncytiotrophoblasts secrete hormones and express efflux transporters, including the multidrug resistance-associated proteins (MRPs) 1 and 5. Understanding the interplay of cyclic nucleotides, efflux transporters, and syncytialization is important because aberrant cytotrophoblast functioning contributes to the pathogenesis of placental diseases and toxicities. In the present study, we sought to determine whether MRP transporters regulate the syncytialization of human placental cells. BeWo b30 trophoblasts were treated with vehicle, MK-571 (25 µM), B-8-Bromo-cAMP (100 µM), forskolin (25 µM), IBMX (200 µM), or their combination for up to 24 hr. Markers of trophoblast cell fusion (glial cells missing homolog 1, GCMI; synectin 2) and hormone secretion (human chorionic gonadotropin, hCGa and hCGb) were quantified by qPCR. Treatment of BeWo cells with MK-571, a pharmacological inhibitor of MRPs, increased intracellular concentrations of cAMP and cGMP by 15- and 19-fold, respectively. Moreover, MK-571 treatment up-regulated cell fusion and hormone secretion markers (GCMI, hCGa, hCGb, synectin 2) by 20-200% under basal conditions. Similarly, MRPI inhibition with MK-571 further enhanced syncytialization and hormone markers following stimulation with the permeable cAMP analog 8-Bromo-cAMP (2- to 6-fold), activation of cAMP synthesis with forskolin (20-160%), or inhibition of cAMP/cGMP breakdown by IBMX (50-150%). In addition, shRNAs were used to generate a stable BeWo B30 trophoblast cell line with reduced MRPI expression. Control and MRPI-knockdown cells were treated with vehicle or 8-Bromo-cAMP for 24 hr. In response to treatment with 8-Bromo-cAMP, the induction of GCMI, hCGa, and hCGb mRNA expression was higher in MRPI knockdown cells as compared to control cells. Furthermore, MRPI-knockdown cells were larger in size (including greater numbers of multinucleated cells) and exhibited decreased staining of the tight junction protein E-cadherin, pointing to enhanced syncytialization. In conclusion, MRP transporters participate in trophoblast cell fusion, which likely represents a novel mechanism regulating placentation and placental toxicities. Supported by F31ES029794, ROI5S020725, T32ES007148, P30ES005022.

Proteomic Profiling of Primary Human Villous Cytotrophoblasts Exposed to BDE-47

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The human placenta serves as the interface between the maternal and fetal compartments and is a target of various environmental stressors. Placental cytotrophoblasts (CTB) play critical roles in implantation, uterine infiltration, and vasculature development with perturbations in CTB function underlying severe pregnancy complications (e.g., preeclampsia, preterm birth). In vitro, primary human villous CTBs differentiate towards an invasive phenotype, representative of their characteristics in vivo. Recently, we demonstrated the utility of this model system to evaluate the toxicological potential of a common flame retardant, brominated diphenyl ether (BDE-47), identifying changes in functional and transcriptomic levels. In this study, we expanded our initial analyses of BDE-47 by profiling the proteome of CTBs using sequential window acquisition of all theoretical mass spectra (SWATH-MS). Protein homogenates were collected from CTBs (2nd trimester, n=60) at 0, 15, 24, or 39h, exposed to BDE-47 (1 or 5µM), vehicle (0.1% DMSO), or media only (control). In total, we commonly identified 3,026 unique proteins across samples. In controls, significantly higher protein expression was observed in 477 proteins (p<0.05), including molecules important in CTB differentiation processes such as metabolism (e.g., PAPPA2), maternal-fetal tolerance (HLA-G), invasion (MMP14), adhesion (ITGA5), vascularization (HSPG2) and extracellular matrix organization (NID1). In a concentration- and time-dependent manner, BDE-47 significantly disrupted expression of 323 proteins, including targets linked with CTB differentiation (e.g., HSPG2) and oxidative stress (e.g., NF-kB). Our results provide a proteomic framework of human villous CTB differentiation in vitro and indicates its sensitivity to developmental toxicants, with the potential to model environmental interactions in the human placenta. Supported by NIH/NIEHS R00ES023846, P01ES022894; US EPA RD883467801.

Myco toxin Zearalenone (ZEA) Induces Toxicity and Alters microRNA Expression in C57Bl/6 Mouse Placenta

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Zearalenone (ZEA) is a major mycotoxin 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 in mice that ZEA diets (20-40 ppm) impaired female fertility. 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 and checked for a vaginal plug the next morning. The day of vaginal plug detection was designated as gestation day 0.5 (D0.5). On D5.5 (post-implantation), the plugged females were 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 fetuses in 40 ppm group, and reduced weight of placentas with live fetuses 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, dilated maternal blood spaces filled with nucleated maternal blood cells, or areas with reduced size. These data indicate disorganized placentae in both ZEA treatment resulting in insufficient fetal-maternal interface for efficient nutrient and gas exchange, which may count for the reduced fetuses 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 epigenetic profiles to regulate gene expression during 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 reveals a unique placental miRNA profile. Interestingly, several of the differentially expressed miRNAs by ZEA treatment were also dysregulated in the placentas from patients with preeclampsia. We continue investigating cellular and molecular mechanisms of ZEA-induced placental toxicity.

Per- and Polynonylalkyl Substances (PFAS) Effects on Mouse Mammary Epithelial Cells

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This study examines both legacy and replacement per- and polyfluoroalkyl substances (PFAS) for effects on the HC11 mouse mammary epithelial cell line. The mouse mammary gland is a sensitive target for the effects of perfluorooctanoic acid (PFOA), therefore PFOA was included as a positive control. HC11 cells were seeded in a 384-well plate at a density of 2,000 cells/well and incubated for 24 hours in RPMI media. Cells were then treated in a blinded fashion with half log concentrations of 20 PFAS (0.95 µM, 3 µM, 9.5 µM, 30 µM, 94.5 µM, and 300 µM) and incubated for 48 hours. During this time, proliferation and cell morphology were measured every 4 hours with an IncuCyte live cell imaging and analysis system. The HC11 cells were tested for cell viability using the CellTitre Glo (CTG) assay 48 hours after treatment. The positive control, PFOA, not only induced cytotoxicity and decreased proliferation rates, but also caused the HC11 cells to become enlarged at the highest concentration tested. Preliminary findings demonstrated that seven PFAS in the current library inhibited proliferation at a Low Effective Concentration (LOEC) of 300 µM (4 µM PFOA), 9.5 µM (n=1), 3 µM (n=1), or 0.95 µM (n=1), and several PFAS had little to no effect on proliferation of mammary epithelial cells. CTG assays estimated 50% Inhibitory Concentrations (IC50) of 299.7 - 8.5 µM, varying for each of the 15 PFAS that had an effect. Proliferation LOEC values were not always consistent with the IC50 values for a given PFAS, presumably due to morphological changes produced by some treatments. This led us to utilize cell count to normalize proliferation and cytotoxicity data. Further tests are being conducted on the 25 remaining chemicals in the library for prioritization of the most active mammary toxicants for future research projects in differentiated HC11 cells and mice. We hope to contribute dose-response information in a PFAS-sensitive target organ for use in risk evaluation of these chemicals.
1527 Impact of a Vermiculite-Nanocellulose Hybrid on Sperm Characteristics, Endocrine Balance, and Redox Status in Male Rats


The safety of vermiculite-nanocellulose (VEN) hybrid on the male reproductive system is not known. Hence we investigated the effects of VEN on the biochemical and hormonal indices of rats. Animals were orally exposed to VEN at 0, 5, 10 and 20 mg/kg body weight for 14 consecutive days. Administration of VEN had no effect on organo-somatic index of the testes. Although Gamma-glutamyl transferase, catalase and superoxide dismutase activities and hydrogen peroxide level remained unaffected, exposure of rats to VEN significantly depleted myeloperoxidase, glutathione peroxidase and malondialdehyde levels in the testes of treated rats, when compared to control. The spermogram of VEN-exposed rats showed a significantly higher sperm count and lower sperm morphology rates than control group. Treatment with VEN also significantly increased circulating concentrations of follicle-stimulating hormone and testosterone levels at the 20 mg/kg dose. Histo-architecture of the testes, in the treatment groups, revealed normal looking Leydig and Sertoli cells. Taken together, our study show that VEN could play an important role in male reproduction in rats, especially in the stimulation of hormononal secretion.

1528 Reproductive Toxic Effect of Cnestis ferruginea (de Candolle) Root Extract in Male Rats


Cnestis ferruginea (Conneraceae) is a shrub used locally as a bacteriolytic and antiinflammatory agent in the southern part of Nigeria. Many antimalaria medicinal plants have effects on the reproductive system. This study was carried out to evaluate the reproductive toxicity of the methanolic extract and purified fractions from the root of the plant in rats. The methanolic extract was administered orally at 500, 1000 and 2000 mg/kg bw to rats weighing 160-220 g in the test groups and distilled water to those in the control group for 5 (acute), 30 (sub-chronic) and 60 (chronic) days. Effects of the extract and purified fractions, on male fertility were assessed by sperm function analyses, testicular weight, mating experiment and histological examination of testes. The reproductive effects of the extract and purified fractions were compared with those of quinine sulphate. Plasma testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) concentrations were determined to elucidate the probable mechanism of action of the extract on male reproduction. Student’s t-test and analysis of variance were used to test for levels of significance. Acute oral administration of methanolic extract resulted in dose-dependent reduction in sperm count, viability, motility and morphology. There were decreases (P<0.05) in plasma testosterone concentration at 2000 mg/kg bw and the weight of testes at 500 mg/kg bw during the 5 day exposure. Prolonged administration of 500mg/kg bw of the extract caused significant reductions in sperm count, motility, viability, and morphology on the 30th and 60th day of treatment. Each of the six chromatographic fractions (1-6) from the extract administered orally (100, 1000 and 2000 µg/kg bw for 60 days) caused significant reduction (P<0.05) in the sperm counts, motility, viability, morphology and testosterone values. The methanolic extract and chromatographic fractions caused reversible degeneration of testes. However, fractions 3 and 4 caused the highest reduction (P<0.05) in fertility, FSH and LH levels. The reproductive effects of fractions 3 and 4, which contain quinolizidine alkaloid, were comparable to those of quinine sulphate. There was recovery from the reproductive and toxicity effects of the extract after 60 days of withdrawal. The results suggest that Cnestis ferruginea possesses reversible male antifertility effects which are due to quinolizidine alkaloids.

1529 Testicular Toxicity of Sub-chronic Low-Dose Methotrexate Exposure in Rat


Methotrexate (MTX) is a widely prescribed drug to treat neoplastic, autoimmune and inflammatory diseases. MTX antagonizes the folate pathway and leads to the inhibition of DNA and RNA synthesis, causing cytotoxicity and genotoxicity in various organ systems. In particular, testicular injury has been observed with MTX treatment in both preclinical models and clinically. The present study established a low-dose subchronic MTX exposure model in the rat with the ultimate goal of identifying distinct sperm RNA expression patterns with the purpose of testing whether these patterns could be used as biomarker panels for testicular toxicity assessment. MTX was administered intraperitoneally (IP) at the dose of 2, 5 and 10mg/kg to adult Fisher rats (n=10) weekly for 13 weeks. The animals were sacrificed one week after the last MTX injection. To characterize the testicular injuries, testes/epididymal weights, sperm count, sperm motility and testes/epididymal histology were assessed. Moreover, total RNA was isolated from epididymal sperm and RNA yield per sperm was calculated by normalizing the total RNA yield to sperm count. Sperm content and morphology were similar among the control and treated groups. MTX treatment at 10mg/kg significantly decreased testes weight. There was a clear dose-related effect of MTX exposure on testicular histopathology, including a loss of spermatocytes in late stages of the seminiferous epithelial cycle and a loss of round spermatids in early stages of the cycle. MTX also caused a seminiferous tubule diameter. Neither retained spermatid heads (RSH) nor Sertoli cell vacuoles were a prominent feature, only being observed in a minority of the high dose rats. Epidydymal round cells consistent with sloughed germ cells were dose-dependently increased. The RNA yield per sperm were not altered by MTX treatment. These data showed that a 13-week exposure to low-dose MTX caused testicular damage indicated by low testes weight and histopathological abnormalities.

1530 Effect of Serum Organochlorine Pollutants (DDTs, HCB and PCBs) Levels on Human Sperm Parameters

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Alarming decline in sperm count worldwide over the past decades is the indication of involvement of many environmental and lifestyle changes around the globe. The process of spermatogenesis is an easy target for disruption by various environmental pollutants. PCBs and DDTs have been studied widely as a causal factor in many diseases. This study was aimed to elucidate the relationship between the serum levels of PCBs, HCB and DDTs with sperm motility and concentration. Blood and semen samples from 70 men of age between 20 to 45 were collected in 3cc glass tube and serum was separated at the spot. Detailed questionnaire and consent forms were filled. Semen samples were used to analyze the sperm concentration and motility. One ml of serum was used for the determination and quantification of PCB congeners, HCB, DDT and its metabolites by using head space solid phase microextraction (HS-SPME-GCMS). SPSS 24.0 was used to analyze the data statistically. Semen analyses revealed that 41.4% of the men tested were normospermic, 37% azoospermic, 11% with oligospermia and 10% were found to be asthenospermic. All samples were found positive for HCB with a mean value of 0.37 ng/ml. All PCBs congeners were significantly correlated with sperm concentration (p<0.05) while no significant correlation was found between PCBs exposure and sperm motility. While for the DDTs, only op’DDT and op’DDE showed significant correlation (p<0.05) with sperm concentration. Results suggest that exposure to organochlorine pollutants can affect sperm production but have no effects on sperm motility. However further studies with high number of samples are needed to confirm the findings.

1531 Intra-testicular Testosterone Levels In Vivo vs. In Vitro

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The measurement of intra-testicular testosterone (T) levels is an important aspect of reproductive drug toxicity assessment and reliable in vitro models for spermatogenesis are needed for minimizing animal use. However, in vitro models have not yet been established since spermatogenesis is a complicated process. We have evaluated an in vitro organ culture system that Sato et al (2011) demonstrated vital mature sperm production. We confirmed mature sperm using their system but until now had not determined if the testis fragments produced T. To examine this, liquid chromatography-tandem mass spectrometry analysis was conducted to measure T levels in the cultured testis fragments (in vitro) and mouse testes (in vivo). We collected C57BL/6J mouse testes at postnatal day (PND) 14, 20, 28, 35 and 40. Mouse testis fragments were obtained at PND 5 and cultured in AlbuMAX™I medium for 35, 42, and 49 days (corresponding to PND 40, 47, and 54). Round spermatids in the testis fragments, that were observed at PND 20 in vivo, were first observed on day 23 of culture (corresponding to PND 28), suggesting germ cell differentiation in vitro is slower than in vivo. T levels in mouse testis were initially detected at PNDs 14 and 20 and increased from PND 28 through PND 40. In contrast, T was detected in tests fragments beginning at days 35 and 42 of culture, reaching a maximum on day 49 of culture. Although T production in...
vitro was delayed and lower than observed in vivo, we confirmed that the cultured testis fragments produced T, which follows a similar temporal sequence to that in vivo. Although further improvements to this in vitro organ culture system are still necessary, these findings suggest that this mouse testis organ culture system could provide a useful tool for future physiological studies. The views presented do not necessarily reflect those of the US FDA.

1533 Ethylene Glycol Monomethyl Ether Exposure Alters Cleavage of tRNA Fragments in Rat Sperm
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Many male reproductive toxicants adversely affect fertility through mechanistic targets in one or more distinct cell types in the testis. Regardless of the primary cell targeted by a testicular toxicant, the common thread among these compounds is impairment of spermatogenesis and/or sperm quality. Ethylene glycol monomethyl ether (EGME) targets a specific germ cell subset, the primary spermatocytes, and leads to germ cell apoptosis at high levels of exposure. We hypothesized that the sperm that developed from EGME-exposed germ cells, and their epigenetic contents delivered to an oocyte upon fertilization, may be compromised. Specifically, we looked at tRNA fragments (tRFs) in sperm, because they have been shown to influence embryonic development. Rats were exposed to 0, 50, 60 or 75 mg/kg EGME for 5 days, and then sperm were collected 5 weeks later. Small RNA sequencing was performed on RNAs isolated from the sperm. The total amount of tRFs relative to library size did not change with treatment; however, the tRFs became longer as a function of treatment, significantly so at 60 and 75 mg/kg. At 60 and 75 mg/kg EGME, there was a shift in the fragment size distribution, with a decrease in peak between 22-27 nucleotides and an increasing peak of 28-33 nucleotides. Using the interactive genome browser, the fragmentation pattern of tRNAs was readily visualized; for example, the tRNA-GlyGCC were mostly 5' fragments which became progressively longer with increasing EGME exposure. Since 5'S-tRNA-GlyGCC has been implicated in the transmission of metabolic disease in mice, these data raise the concern that EGME disruption of normal tRNA fragmentation results in altered epigenetic contents of sperm and potential effects on embryogenesis.

1534 Sperm RNA as a Biomarker of Semen Quality across Species

Evaluation of human reproductive risk from environmental and pharmacological chemicals includes extrapolation from data collected using animal models. Since there is an urgent need to identify novel, non-invasive and reliable approaches to monitor the effects of environmental and therapeutic agents on male reproductive health, the present study investigated associations between spermatozoal RNA content and semen quality across species. Semen specimens were collected from men aged 18 to 55 years undergoing male factor infertility evaluation, treatment for autoimmune arthritis with methotrexate, and from proven fertile men. Semen samples were analyzed according to World Health Organization 2010 criteria. Rat and mouse semen specimens were collected from control animals via repeated needle punctures of the cauda epididymides. Sperm large and small RNAs were extracted after somatic cell lysis. The association of human spermatozoal large or small RNA contents with semen quality and lifestyle factors was evaluated using a generalized additive model and one-way ANOVA. Cross-species mRNA sequencing analysis was also performed, and large and small RNA contents per sperm were evaluated using one-way ANOVA with post-hoc Tukey test. In humans, sperm total count was inversely associated with spermatozoal large RNA content. Sperm motility was inversely associated with spermatozoal large and small RNA contents while large RNA content per sperm was significantly higher in semen samples showing abnormal numbers of round cells. Alcohol consumption was strongly associated with increased large RNA amount per sperm. Patients treated with a known testicular toxicant, methotrexate, showed 25 times more spermatozoal large RNA content than the control fertile group. Mouse sperm samples showed significantly increased spermatozoal large RNA content compared to humans. No differences were identified across species in small RNA content per sperm. In conclusion, spermatozoal large RNA content has the potential to predict sperm abnormalities and male reproductive risk following environmental and pharmaceutical chemical exposure in humans. mRNA sequencing data analysis is underway to identify sperm transcriptional similarities and differences among humans, rats, and mice.

1536 Toxicity of 1,4-Dinitrobenzene on TM4 Sertoli Cells: Its Influence on Mitochondria, Cell Monolayer, Androgen Binding Hormone and Stem Cell Factor Genes
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1,4-Dinitrobenzene (1,4-DNB) an industrial and environmental toxicant has been shown to affect the testes but the mechanism of action remains unclear. Sertoli cell plays a key role in spermatogenesis; however, the toxic effects of 1,4-DNB on Sertoli cells have not been studied. The hypothesis of this study is to unravel the mechanism of action of 1,4-DNB induced TM4 Sertoli cells toxicity. TM4 cell line, derived from mouse Sertoli cells were obtained from American Type Culture Collection (ATCC), USA and were exposed to 1,4-DNB (10, 30, 50 and 100 µg/ml) for 4 and 24 h and its activities on cell viability, activity of γ-GT enzyme, apoptosis, ROS and mitochondrial membrane were assessed. The present study showed that exposure of TM4 Sertoli cells to 1,4-DNB decreased the activity of γ-GT enzyme, inhibited cell viability, promoted apoptosis, mitochondrial membrane potential impairment and reactive oxygen species production. Findings from this study showed that exposure to 1,4-DNB destroyed tight junctional structure in Sertoli cell monolayers as evidenced by a decline in TEER value. The results from mRNA expression levels indicated that 1,4-DNB caused up regulation of androgen binding hormone and stem cell factor genes. In conclusion, 1,4-DNB have great potential to increase oxidative stress, inhibit spermatogenesis, expose Sertoli cells to external insults thereby compromising the blood-testis barrier and contributing to male-mediated reproductive toxicity.

1537 Evaluation of Multiple Generations of Rats following Developmental Bisphenol AF Exposures
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Bisphenol AF (BPAF) is a fluorinated analogue of Bisphenol A, used in the manufacturing of consumer products. This study determined reproductive and endocrine-related outcomes in Sprague Dawley rat offspring following pre and post-natal exposure to BPAF. Timed-pregnant dams were randomly assigned to dose groups (Vehicle control; VC; n = 16; BPAF: n = 8 or 9). Dams were gavaged, twice daily starting on gestation day (GD) 6 through postnatal day (PND) 21 in groups that were blindly allocated, and body weight (BW) adjusted daily; 0, 0.25, 1.0, 2.5, or 25.0 mg BPAF/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, 4, 4, 4, 4). On PND 4 and 21, one pup/sex and their respective dams (PND 21) were euthanized and biological samples were collected for future transcriptomic and internal BPAF dose. All remaining female pups were orally gavaged with same doses and evaluated for pubertal timing. F1 females were mated with VC F1 males to produce F2 generation and early life data were collected. Mammary glands and ovaries were collected from F1 females for histological evaluation. The dam’s percent BW gain during gestation was significantly lowest in the highest dose group (p < 0.01) compared to VC. The gestational length and fetal loss during gestation (= total implantation sites – total pups born) were not affected. The loss of pups from birth until PND 4 (neonatal 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 p < 0.01; respectively). 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). Conception rate was unaffected in the F1 females exposed to BPAF. In F1 transgenic fetal liver fibroblasts was higher in the high dose group (p < 0.01) and primary follicle counts were lower in dose groups 0.25, 1.0, and 2.5 mg BPAF/kg BW (p = 0.012, 0.02, and 0.14, respectively). Mammary glands exhibited higher stromal density in non-pregnant F1 females (p = 0.017). Data suggest that exposure at the highest dose level of BPAF tested altered several contraceptive endpoints in F1 female offspring. The persistent effects on body weight and elevated pup loss may be related to the potential disruption of normal hormonal activities. On-going studies will determine internal doses associated with the noted biological effects as well as transcriptomic changes in affected tissues.
The toxicological potential of cyclamen aldehyde, a widely used flavor and fragrance ingredient, was examined by conducting an enhanced OECD 415 one-generation reproduction study. Sprague Dawley rats (25 rats/sex/dose) were gavaged with cyclamen aldehyde at doses of 0, 25, 75, or 150 mg/kg/day in corn oil. Treated male and female rats were cohabitated with untreated female and male cohorts, respectively. F1-generation rats were not directly dosed but instead were exposed to the test material in utero during gestation and through postpartum maternal milk. High-dose P-generation males were reported to be infertile following mating with untreated females. The absence of motile sperm and altered sperm morphology were observed in 13/25 mid-dose and all of the high-dose group rats. Grossly visible masses were reported on one or both cauda epididymides among high-dose P-generation males. Microscopic examination of the epididymis revealed moderate to marked sperm granulomas. Only 1 untreated female rat assigned to mate with high-dose male rats became pregnant but was unable to deliver a litter. No apparent effects were observed in the development of pups up to 75 mg/kg/day. Thus, the NOAEL for male reproductive toxicity was considered to be 25 mg/kg/day. Significant decreases in implantation sites, number of delivered pups, pup survival and litter size, and increased pup mortality were observed among high-dose P-generation females. Ovary weights among high-dose females were decreased and the uterus weights of mid- and high-dose females were increased. In utero and lactation exposure to cyclamen aldehyde caused a significant reduction in pup body weights and body weight gains among mid- and high-dose group pups. Thus, the NOAEL for female reproductive toxicity and developmental toxicity was considered to be 25 mg/kg/day. Later studies indicate that the effect in rats is not relevant to human health, since rats selectively metabolize cyclamen aldehyde to its reproductive toxic metabolite responsible for effects reported in the present study. Furthermore, rabbits were observed to be the more relevant species to model human toxicity, as rabbit metabolism of cyclamen aldehyde were comparable to humans, and showed an absence of reproductive toxicity that were found in rats.

Biphenyl (BP) is an aromatic hydrocarbon with a rich toxicological dataset that has recently been tested in an EOGRTS, which included cohorts 2 (developmental neurotoxicity) and 3 (developmental immunotoxicity). Although limited in vitro data exists with BP, it selectively metabolizes cyclamen aldehyde to actuate certain endocrine receptors, these metabolites are transient in vivo and are quickly detoxified by phase II conjugation reactions. Moreover, ToxCast™ endocrine receptor assays do not suggest BP acting via an endocrine disruption (ED) mode-of-action (MoA). The EOGRTS has been conducted under the European REACH programme to assess various endocrine parameters as well as effects on nervous and immune system during critical windows of development. CritCD(SD) rats were fed diets containing 0, 300, 1000, and 2800 ppm BP throughout the entire study. Endocrine parameters evaluated included estrous cyclicity, ovarian follicle counts, reproductive indices, sperm parameters, anogenital distance, nipple retention, puberty onset, thyroid hormone, thyroid/pituitary/adrenal/reproductive organ weights and histopathology of these tissues. Two incidences of dystocia in each of the low and mid dose P1 dams were observed, resulting in breeding of the second generation to clarify these findings. However, no incidences of dystocia occurred in P2 BP-treated dams, and one incidence occurred in a P2 control dam, demonstrating that this finding was sporadic and unlikely to the treatment. The parents and offspring of both generations showed no evidence of treatment-related effects on reproductive parameters or on the estrogen-, androgen-, or thyroid-related endocrine pathways at any BP dose. There was no evidence of structural or functional changes in the nervous or immune system due to BP exposures during critical windows of development. Overall, it is concluded that BP, at doses up to 2800 ppm, does not act via an ED MoA nor does it affect immune or nervous system development.
Combined Fertility and Embryofetal Developmental Toxicity Study of Cytisine in Rats

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Cytisine ([1R,5S]-1,2,3,4,5,6-hexahydro-1,5-methano-8H-pyrido[1,2,3,4]diazicacin-8-one) is a plant alkaloid used in Eastern Europe for smoking cessation. Through its activity as a partial agonist of α4β2 nicotinic acetylcholine receptors, cytisine may attenuate symptoms of nicotine withdrawal. This study was performed to assess the potential reproductive and developmental toxicity of cytisine in rats. Groups of 22 CD rats/sex received daily oral (gavage) administration of cytisine (in sterile water) at doses of 0 (vehicle control), 0.4, 2, or 10 mg/kg/day beginning 4 weeks (males) or 2 weeks (females) prior to mating. Dosing was continued in males until Day 72 and in females until scheduled cesarian sections on Gestation Day 20. Six additional rats/sex/cytisine group were included for toxicokinetics (TK). Endpoints included plasma drug levels; TK; in vivo toxicity; estrus cyclicity; mating/fertility/fecundity; reproductive parameters and organ weights; semen analysis; fetal body weights; and fetal examinations to identify developmental toxicity. Plasma levels of cytisine (AUC) were significantly greater in all dose groups; systemic exposure was generally higher in females than in males. In the high dose group, T1/2 was 5.1 h in males and 6.6 h in females. No mortality or treatment-related gross toxicity was seen in any rat receiving cytisine. Statistically significant decreases in body weight gain were seen in both sexes in the high dose group (10 mg/kg/day); sporadic signs of food consumption were seen in high dose (10 mg/kg/day) and mid dose (2 mg/kg/day) groups. Cytisine had no effect on estrus cyclicity; sperm counts, morphology, or motility; reproductive organ weights in either sex; mating indices; or other parameters associated with fecundity or reproductive function. Fetal body weights were comparable in all groups; no evidence of cytisine developmental toxicity (increased incidences of malformations or variations) was identified in external, visceral, cephalic, or skeletal evaluations of fetuses. Cytisine doses of up to 10 mg/kg/day induced no evidence of developmental or reproductive toxicity in rats; cytisine toxicity was limited to reduced body weight gain in the high dose group (10 mg/kg/day). Supported by National Center for Complementary and Integrative Health through HHSN261201600015I from NCI.

Interspecies Comparison of Embryo-Fetal Data among Control Groups of Sprague-Dawley Rats, New Zealand White Rabbits and Gottingen Minipigs

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Understanding species-dependent differences in relative incidence of spontaneous variations and malformations is important for reproductive and developmental safety assessment. The objective of this evaluation was to compare litter parameters and external, visceral, and skeletal malformations and variations across species in the Sprague-Dawley rat, New Zealand White rabbit and Gottingen minipig. Pregnant female rats (n=871), rabbits (n=465) and minipigs (n=70) from vehicle control groups were included in the analysis equating to 11460 rat, 4486 rabbit and 378 pig fetuses collected at term by cesarean section. Pre-implantation loss was more frequent than post-implantation loss in the rat and rabbit, whereas the opposite was observed in the minipig (rat: 10.9%, 4.7%; rabbit: 13.8%, 8.1%; minipig: 7.6%, 10%). Several external and visceral malformations and variations such as domed head, skin discolaration, cleft palate, abdominal edema, and anal atresia were observed in all three species. Visceral malformations of the heart and the major blood vessels were remarkably more frequent in the minipig: Ventricular and atrial septum defects were observed in 1.9 and 2.1% of the minipig and 0.2 and 0% in the rabbit whereas they were not observed in any rat fetuses evaluated in this study. Our results indicate that the minipig presents a higher spontaneous incidence of heart malformations consistent with humans, as congenital heart defects are the most common types of birth defects in humans (approximately 1% of births). A thorough understanding of the similarities and differences in spontaneous malformations in different species is important to interpretation of embryo-fetal development studies.
Automation of the In Vitro Micronucleus Assay for Genetic Toxicology Testing Using Imaging Flow Cytometry

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The in vitro micronucleus (MN) assay is a well-established method for evaluating genotoxicity and cytotoxicity. MN are formed from whole chromosomes or fragments that lag behind following metaphase and are excluded from the main nuclei following division. The presence of MN is indicative of chromosomal mutations and their quantification can be used as an endpoint in genotoxicity testing. Manual microscopy is typically used to perform the MN assay but is laborious and suffers from low throughput and inter scorer variability. Automated methods, including slide-scanning microscopy and conventional flow cytometry have been developed, but these methods have drawbacks including lack of cytoplasmic visualization and the inability to visually confirm the legitimacy of MN. Imaging flow cytometry (IFC) has the potential to overcome these limitations as it combines the speed and rare event capture capability of flow cytometry with the high resolution imagery obtained by microscopy. A method to perform the in vitro MN assay on the ImageStreamX MkII (ISX) has been developed. Images of several thousand mono-, bi- and polynucleated cells can be captured and automatically identified in the Image Data Exploration and Analysis Software (IDEAS®). Human TK6 cells were exposed to two clastogens and two aneugens (Mitomycin C, Methyl Methanesulfonate, Colchicine and Vinblastine Sulfate) and one negative control (Mannitol) for 3 hr. Following a 24 hr recovery period, cells were harvested, stained with Hoechst and run on the ISX. Several thousand images of micronucleated mono- and bi-nucleated cells as well as polynucleated cells were captured on the ISX, automatically analyzed, sorted and enumerated in IDEAS® in just a few minutes, allowing for the evaluation of both genotoxicity and cytotoxicity. Statistically significant increases in MN frequency with increasing dose were observed for all compounds tested when compared to solvent controls, except for Mannitol, as expected. The high throughput nature of the ISX overcomes many of the challenges of conventional techniques. More samples can be collected and scored, improving the statistical robustness of the MN assay and all collected imagery can be stored in dose-specific data files for re-evaluation. This work represents the first step towards the development of a fully automated approach for performing the in vitro MN assay to assess cytotoxicity and genotoxicity using IFC.

Benchmark Dose Modeling of In Vitro Genotoxicity Data from the Mouse Lymphoma Assay

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Applied genetic toxicology is now changing to quantitative risk assessment from qualitative hazard identification. Recently, quantitative analysis with point of departure (PoD) metrics and benchmark dose (BMD) modeling have been applied to in vitro genotoxicity data. Two software packages are commonly used for BMD analysis. In the previous studies, we conducted the mouse lymphoma assay to evaluate the mutagenicity of four pipeline nitrooxides having various substituent groups on the 4-position of the pipеридине ring and six cigarette whole smoke solutions (WSSs) prepared by bubbling machine-generated whole smoke. In the present study, we performed quantitative dose-response analysis on these genotoxicity data using the PROAST software and the US EPA’s BMD software (BMDs) and evaluated the inter-platform quantitative agreement in genotoxic potency estimates. We calculated the BMDs for 10%, 50%, 100% (i.e., 2-fold increase), and 200% increases over concurrent vehicle controls in order to better discriminate between the dose-responses, along with their BMDLs (the lower 95% confidence interval of the BMD) and BMDUs (the upper 95% confidence interval of the BMD). The BMD values and rankings estimated using the US EPA’s BMDs were reasonably similar with those calculated using the PROAST. These results indicated that both software packages are suitable for dose-response analysis using the mouse lymphoma assay, and that the BMD modeling results from these two software packages are comparable for rank ordering mutagenic potency.

Is It Appropriate to Set 5% CD71+ Reticulocytes of Control as the Toxicity Limit in the Flow Cytometry-Based Rat Blood Micronucleus Assay?


Since the 2014 version of OECD testing guideline 474, the required number of immature erythrocytes scored has increased from 2,000 to 4,000 in microscopy-based micronucleus (MN) assay; the throughput of the traditional MN assay becomes an issue, moreover, manually scoring slides for MN can be somewhat subjective and dependent upon staining quality. Fortunately, automated analysis and rat blood are formally accepted for in vivo MN assay by the current version of ICH 52/12 and OECD TG 474. The in vitro flow cytometry (FCM)-based method for evaluating MN frequencies in erythrocytes shows great potential for improving sensitivity, reproducibility, and throughput compared to the traditional method. The OECD TG 474 guideline defined that the proportion of immature erythrocytes to total erythrocytes in treated animals should not be less than 20% of control proportion by microscopy-based method and approximately 5% of control proportion when scoring CD71+ immature erythrocytes by FCM method. However, we had experienced that this limit setting may cause false positive. We would like to share a case study to attempt to draw attention while using the bone marrow toxicity limit in the FCM-based rat blood MN assay. In the original paper (LeBaron et al, Environ Mol Mutagen, 2012), the rational for setting the 5% target value is that %RET cytotoxicity evaluated in blood showed greater responsiveness than %PCe endpoint in bone marrow, which was consistent with majority compounds tested in our lab. However, all compounds selected in the paper are clastogens, which may obscure the effect on MN induced by toxicity. In our case study, the test article is an oral drug candidate for treating arthritis. It was tested negative in the Ames assay up to 5000 µg/plate, and in chromosomal aberration test in CHO-WBL cells up to cytotoxic concentrations. In the acute rat MN assay, 2-fold MN increases were observed only at the high dose group compared to the control in males, 55.75% and 87.64% RET reductions were observed at the mid and high doses respectively. In the repeat MN assay, the percentage RET reductions in the high dose group was lowered to 53.55%, the assay met the OECD valid criteria and no MN increase was observed at any dose. Our data suggests the positive data should be interpreted cautiously at toxic dose level, especially when above 80% RET reduction. We can’t clearly conclude that the toxicity will induce false positive in rat blood MN assay, but at least the dose level selection might have effects on any final call, even if both scenarios meet the OECD valid criteria.

Low Equimolar 90-Day Exposure of Fungicides Carbendazim and/or Thiram in Drinking Water Caused Persistent Genotoxicity

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In Nepalese agriculture, carbendazim and thiram are the most commonly used fungicides to prevent Botrytis gray mold disease of chickpea, the major source of protein in the Nepalese diet. To our knowledge, none of these fungicides cause DNA damage either acutely by high doses or in 28 days by injection, which leads to distinct morbidity signs. Six-week-old male Swiss Webster mice (N= 5 per group) were exposed to untreated drinking water or 40 µM of either fungicide alone or together for 90 days to test for chronic effects at levels below water solubility limits to observe synergy, additivity or lack of effects. One group was analyzed at 90 days and one after a 45-day recovery period to check for persistence of damage. A unique more environmentally relevant result was that water or food intake and body weights throughout the experiment, and liver weights and kidney weights at sampling time were unaffected with no observed morbidity or aversion to intake at these concentrations. White blood cell viability was assessed at ~97% by Trypan blue exclusion following RBC lysis. Comet assay results from whole blood (WBCs) under alkaline conditions using a commercial kit at 90-day exposure or 45 days thereafter led to almost identical results. Controls mean tail DNA± SD were assessed (50 comets) at 9.76±6.30 at 90 days or 7.01±4.74 at 135 days (90 ±45) similar to undamaged CCO cells provided with the kits (8.9±6.1 and 9.7±6.3 for kits used at 90 and 135 day, respectively). Carbendazim, thiram or combination exposure resulted in similar significant (P<0.05) damage with mean tail DNA± SD of 33.0±20.4, 30.5±17.2 and 27.8±22.1, respectively, similar to commercially provided CC1 cells slightly damaged with etoposide (32.4±17.8). After a 45-day recovery period this same order of treatments caused mean tail DNA± SD of 27.5±12.9, 29.3±15.4 and 32.0±12.8, respectively, which again was not significantly different from CC1 cells (34.8±20.2). It appears when mice are given a chronic exposure of the combination of both fungicides in drinking water, there was no synergy or additivity, but rather a possible competition for absorption or toxicity. However, the most disturb-
ing result was the lack of any noticeable repair after an extended recovery period indicating that chronic low-level exposure in drinking water leads to persistence of genotoxic damage.

1550 Quantitative Analysis of Genotoxicity and Cytotoxicity of the Nitroxide Radical 2,2,6,6-Tetramethylpiperidin-1-oxyl (TEMPO) and TEMPO Derivatives

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2,2,6,6-Tetramethylpiperidin-1-oxyl (TEMPO) is a low molecular weight nitroxide and stable free radical. TEMPO has been demonstrated to be cytoxic and mutagenic in mammalian cells. It remains unclear whether or not TEMPO derivatives induce similar cytotoxic and mutagenic effects as does TEMPO. In this study, we investigated the cytotoxicity and genotoxicity of TEMPO and three of its derivatives (4-hydroxy-TEMPO, 4-oxo-TEMPO, and 4-methoxy-TEMPO) in mouse lymphoma L5178Y Tk−/− cells. As results, all tested nitroxides induced dose-dependent cytotoxicity and genotoxicity. The cytotoxicity and genotoxicity were generally greater in the presence of exogenous metabolic activation (S9) except 4-hydroxy-TEMPO. The results showed that TEMPO was more cytotoxic than the three TEMPO derivatives. Tk mutants induced by all four nitroxides possess different loss of heterozygosities patterns compared to those of the untreated control. The four nitroxides caused concentration-dependent increases in mutant frequency and DNA damage as measured by the mouse lymphoma assay (MLA) and comet assay, respectively. Using a multi-endpoint DNA damage pathway assay, TEMPO and its derivatives were classified as clastogens, involving strand breakage and large alterations to DNA. Benchmark dose quantitative approaches indicate that the most potent nitroxide was 4-oxo-TEMPO in the MLA and TEMPO in the comet assay, respectively. In conclusion, TEMPO, parent compound, is relatively more cytotoxic and genotoxic than the different TEMPO derivatives. Quantitative dose-response approaches of the genotoxicity data were useful for rank ordering the genotoxic potencies of structurally similar compounds.

1551 Assessment of the Mutagenic Potential of Para-Chloroaniline and Aniline in the Liver, Spleen and Bone Marrow of Big Blue Rats with Micronucleus Analysis in Peripheral Blood

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Spleenic tumors have been reported in rat cancer bioassays with para-chloroaniline (PCA) and aniline. Development of these tumors is hypothesized to be due to hematotoxicity via the formation of methemoglobin (MetHb) and not direct DNA reactivity. To evaluate the mode of action for tumor formation a transgenic rodent in vivo gene mutation assay in Big Blue TgF344 rats was performed with parallel micronucleus analysis in peripheral blood. Male rats were gavaged daily for 28 days to 0.5, 15, and 60 mg/kg PCA and 100 mg/kg aniline, the basemolecular structure of PCA. On test day 10 the 60 mg/kg PCA dose was reduced to 30 mg/kg due to toxicity. On test day 4 and 29 peripheral blood micronucleus analysis was performed and on test day 29 clinical chemistry, hematology, and MetHb measurements were taken. At study termination, on test day 31, spleen (site of tumorigenicity), bone marrow (site of hematotoxicity), and liver (control tissue) were analyzed for clt transgene mutant frequency (MF). Repeat gavage exposure to PCA and aniline for 28 days did not produce an increase in clt transgene MF in analyzed tissues. An increase in micronuclei was seen at both time points at ≥15 mg/kg PCA and 100 mg/kg aniline. At the same dose levels, significant reductions in red blood cells, increases in absolute reticulocytes, and increased levels of MetHb were observed. Together these results support that generation of micronuclei and tumorigenically following exposure to PCA and aniline is due to compensatory mechanisms (e.g. increased cellular turnover) and not direct DNA reactivity.

1552 Presence of a Genotoxic Impurity, 2-Chloro-N,N-Dimethylaminoethylenenitrilechloroformide in Aroclor 1254-Induced S9 Fraction and Possible Relevance to Differential Frequencies of Spontaneous Revertants in the Presence vs. Absence of S9 Activation System


Historically, we have observed notable differences in the frequencies of spontaneous revertants between the activated and nonactivated conditions in Ames assay with the frequencies being notably higher for all strains in the presence of S9 enzyme mediated metabolic activation. As the S9 fraction and the cofactors added to the test mixture are different between the two conditions, the response is attributable to either the S9 fraction and/or cofactors. As a range of concentrations of S9 are used by different laboratories for regulatory submissions, it is important to evaluate the contributions of various components of the testing conditions in order to avoid false positives. A range of S9 concentrations such as 1.0, 2.5, 5, 10 and 20% combined with appropriate amounts of cofactors, were tested using TA 100 strain. There was a steady increase in the frequency of revertants across the range with a maximum increase of approximately 40% occurring at 20% concentration. In order to characterize the qualitative and quantitative differences in the profiles of chemical compounds between S9 extracts from Aroclor-induced and uninduced rats, an analytical evaluation of the same was conducted using HPLC/MS method. The results showed the presence of a genotoxic impurity, namely 2-Chloro N,N-dimethylaminoethylenenitrilechloroformide, in multiple batches of S9 preparations at significant detection levels. 2-Chloro N,N-dimethylaminoethylenenitrilechloroformide, acknowledged to be a process impurity in the manufacture of several chemicals and shown to have structural alerts for mutagenic and carcinogenic potential by QSAR models was not present in uninduced S9 fraction preparations. It can be concluded that 2-Chloro N,N-dimethylaminoethylenenitrilechloroformide impurity is specific to S9 preparations made from animals treated with Aroclor only. These findings are of high significance because the additive and/or synergistic effects with the test compounds could result in the generation of false positives in Ames assay and suggest that the components of the test system used to evaluate mutagenicity in the activated condition should be carefully selected. 1Prashant B. Zate et al (2017), Confirmation and Quantification of Genotoxic Impurity 2-Dimethylaminomethyl chloride hydrochloride (DMC HCl) by GCMS in Chlorpheniramine/Chlorphenamine Maleate, IOSR Journal of Applied Chemistry 10:21-26.

1553 The Effect of Ingredients on the In Vitro Mutagenicity of the Gas Phase from a Heated Tobacco Product


A heated tobacco product has been developed which reduces exposure to toxicants compared to cigarette smoke. The tobacco is heated to 250°C to avoid combustion. Flavour ingredients are added to the tobacco. Their stewardship included in vitro tests of the product’s aerosol emission to measure the effect of added flavour ingredients on the emission’s genotoxicity. The aerosol has a particulate and a gas-vapour phase. As published elsewhere, the flavour ingredients tested had no effect on particulate phase genotoxicity. This poster summarises the results for the gas-vapour phase. The test articles were gas-vapour phase extracts (GVP) from the heated tobacco product with and without flavour ingredients and GVP from a reference cigarette. Conditioned tobacco products were machine-puffed using Health Canada puffing parameters. The emissions were drawn through Cambridge filter pads to remove the particulate phase. GVPs were collected in an ice-cold aqueous buffer and tested within three hours. GVP concentration was expressed as the equivalent concentration of TPM. The in vitro tests were for bacterial mutagenicity (Ames test) and mammalian mutagenicity (mouse lymphoma assay, MLA). All tests were conducted according to OECD guidelines in an independent laboratory complying with GLP. Test results were valid in terms of concurrent control responses and historical ranges. The reference cigarette GVP was cytotoxic in the Ames test and mutagenic in one of the five bacterial strains (TA100 ± S9). At concentrations up to 5000μg TPM equivalent per plate, the GVPs from the heated tobacco products were not cytotoxic or mutagenic. All three GVPs were cytotoxic and mutagenic in the MLA. GVP from the heated tobacco products only induced mammalian mutation at concentrations approximately ten times higher than that of the reference cigarette. Adding flavours to the heated tobacco product had no effect on the in vitro mutagenicity of its GVP.
Genotoxicity Evaluation of α, β Unsaturated Aldehyde Class of Fragrance Materials in the Alternative Chicken Egg Genotoxicity Assay (CEGA) Compared to the Results with Other Regulatory Approved In Vitro and In Vivo Genotoxicity Assays

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The genotoxic potentials of three α,β unsaturated aldehyde fragrance materials, 2-phenyl-2-butenal, nona-2 trans-6-cis-dienal and 2-methyl-2-pentenal, were assessed in the Chicken Egg Genotoxicity Assay (CEGA), using the comet assay to detect hepatic DNA strand breaks. These compounds were selected for testing based on their chemical structures together with the results in regulatory approved in vitro and/or in vivo genotoxicity studies conducted according to OECD guidelines. Groups of at least 10 fertilized chicken eggs received 3 daily injections of either vehicle, positive control (quinolone), or test substances at various dose levels, on days 9 to 11 of incubation. Eggs were terminated 3 hours after the last dose, and livers were collected for analyses. Three tested fragrance materials did not produce statistically significant DNA strand breaks when compared with the solvent control, thus, they were negative in the comet assay, whereas the positive control produced statistically significant positive results. Additionally, when compared to the in vitro in vivo test outcomes for this class of materials, CEGA results were in 100% concordance with those of the in vivo genotoxicity studies. Results, however, differed from in vitro tests, which can be explained by different endpoints measured in the egg model and higher rates of false-positive results in the in vitro testing systems. Thus, our results support the conclusion that CEGA can be considered as a potential animal alternative testing strategy for assessment of genotoxic potential of fragrance materials, which may generate false positive outcomes in in vitro test systems.

3D Skin Micronucleus and Comet Assay as an Animal Alternative Model for Genotoxicity Positive Materials in Traditional In Vitro Studies: A Case Study on p-Methoxycinnamaldehyde

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Evaluation of cytogenetic damage in the 3D primary human skin model offers a biologically relevant in vitro approach to assess genotoxicity following dermal exposures. The 3D Reconstructed Skin Micronucleus (RSMN) and the 3D Full Thickness Human Skin Comet assays provides an animal alternative follow-up testing for materials that are positive in the traditional in vitro assays. p-Methoxycinnamaldehyde, a fragrance material, was evaluated in multiple assays to assess its genotoxic potential. The BlueScreen™ assay was conducted as an initial screen in the presence and absence of metabolic activation (S9), which resulted in a positive outcome. In the Ames assay, the material produced a positive result only in TA100 strain in the presence of S9 and negative in all other strains in the presence or absence of S9. In the in vitro micronucleus assay, the material was considered positive for inducing micronuclei in the presence and absence of S9. In the in vivo Micronucleus and Comet Assays, p-methoxycinnamaldehyde did not induce significant increase in the micronucleated reticulocytes or DNA damage in liver, when compared to the concurrent vehicle controls. To follow-up the positive Ames and in vitro micronucleus assay results and to compare the in vivo assays, the 3D Skin Comet and Micronucleus Assays were conducted. The results indicate that p-methoxycinnamaldehyde was negative for the induction of micronuclei and DNA damage in the RSMN assay in EpiDerm™ and in the 3D Full Thickness Human Skin Comet assay, respectively. Based on the results from the 3D skin models, the material was considered to have no genotoxic potential.

Re-evaluation of Genotoxicity Data for 4,4′-Methylene Dianiline to Improve Data Quality for Hazard Assessment

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4,4′-methylene dianiline (MDA) has been assessed and classified by different authorities as a carcinogen and mutagen [IARC (1986), EU (1993)] primarily based upon studies conducted by the NTP (1983) that demonstrated carcinogenicity. The underlying Mode of Action (MoA) of tumor formation is still not completely understood; however for risk assessment, a genotoxic linear non-threshold approach is assumed. Recent recommendations from OECD, US FDA, ECVM and IWGT on the conduct and interpretation of genotoxicity test data provide a framework to systematically re-evaluate genotoxicity data to improve the reliability and quality of the underlying data for hazard assessments. This approach was exercised with MDA data. Studies were identified via a literature search of publicly available records. Only assays from primary literature were included. A total of 28 genotoxicity assays were re-evaluated and re-scored according to protocols outlined in Klimisch et al. (1992) using current recommendations for genotoxicity testing and data interpretation. The re-evaluation reveals that many of the in vitro mammalian cell assays have shortcomings when compared with current recommendations, like artificial test conditions (inappropriate high cytotoxicity, pH-value shift) and insufficient documentation of test results. Insufficient documentation and excessive toxicity above the maximum tolerated dose (MTD) was also found for some of the in vivo assays. In spite of the shortcomings of some of the in vitro and in vivo test systems which would likely be excluded from hazard assessment; there is sufficient evidence from reliable studies to support the conclusion that metabolically-activated MDA is genotoxic. Findings from well conducted in vivo tests [Pig-a gene mutation assay (Sanada et al. 2014), micronucleus assay (Sanada et al. 2015)] suggest that MDA genotoxicity may be related to a cumulative effect. Thus, repeated dosing studies may be more appropriate to investigate genotoxic effects of MDA versus the standard, single high-dose approach. In conclusion, it might be beneficial for hazard assessments to re-evaluate existing data in light of current knowledge for endpoints like genotoxicity. This will improve the data quality of hazard assessments and gain a better understanding of the underlying MoA. In the case of MDA, such a re-evaluation points to a cumulative effect of MDA genotoxicity, which can be used to more effectively design and conduct studies on this endpoint.
An outbreak of bladder cancer was reported recently among workers who handled aromatic amines in chemical factories in Japan, and aromatic amines have been used for long time there. All of the ten cases of cancer had the records of exposure to o-toluidine, a known human carcinogen classified into Group I chemical by the International Agency for Research on Cancer, and nine cases were also exposed to 2,4-dimethylaniline (2,4-DMA). Information about the genotoxicity and carcinogenicity of 2,4-DMA is limited and inconsistent. The International Agency for Research on Cancer classifies 2,4-DMA as a Group 3 chemical, indicating no evidence of carcinogenicity to humans. Our study aimed to clarify the genotoxic effects of 2,4-DMA and its possible contribution to the occurrence of occupational bladder cancer. Human urothelial (1T1) and hepatocyte (WRL-68) cells were treated with 2,4-DMA at different concentrations for 1-24 hr, and phosphorylated histone H2AX (γ-H2AX), a marker of DNA double strand breaks, was detected by western blot and immunofluorescence staining. To explore the mechanism underlying the genotoxic effects, reactive oxygen species (ROS) production following 2,4-DMA exposure and the mediation of CYP2E1 were evaluated. It was shown that 2,4-DMA generates γ-H2AX in a dose-dependent fashion in both cell lines. The double-strand breaks formed in 1T1 cells after 2,4-DMA treatment was confirmed by the bisbenzimide staining fluorescence electrophoresis. In the mechanistic investigations, we found that 2,4-DMA induces intracellular ROS, an effect clearly attenuated by disulfiram, a strong inhibitor of CYP2E1. Furthermore, CYP2E1 inhibitors and the antioxidant, NAC, also attenuated γ-H2AX generation following exposure to 2,4-DMA. Our results suggest that γ-H2AX is formed following exposure to 2,4-DMA via ROS produced by CYP2E1-mediated metabolism. Continuous exposure to genotoxic aromatic amines over a long period of time may have contributed to the development of bladder cancer. Our results provide insights into the carcinogenicity risk of 2,4-DMA in occupational bladder cancer outbreaks at chemical plants in Japan.

O€\textsuperscript{6}-alkylguanine-DNA-alkyltransferase is a constitutively expressed enzyme in E. coli that is encoded by the ogt gene. Its main function is to repair DNA damaged by alkylation, a common pathway for genotoxicity. To gain greater insight into its DNA repair function, we have exposed wild-type (WT, MG1655) and ogt-deficient (JW1329-1) strains of E. coli to the DNA alkylating agents ethyl methanesulfonate (EMS) and N-ethyl-N-nitrosourea (ENU). Intercellular genetic variance, a next generation sequencing analysis that reliably identifies mutations by comparing sequencing datasets from colonies/clones derived from single cells, revealed that ogt deficiency led to greater levels of DNA damage. EMS exposure generated G:C→A:T transitions almost exclusively, while ENU exposure generated G:C→A:T and A:T→G:C transitions in equal amounts. For both EMS and ENU, the mutation spectra were unchanged between WT and ogt-deficient mutants. These data suggest that ogt plays an important role in DNA repair, and it repairs mutagenic ethylation products at G:C and A:T base-pairs equally well.

Commercially purchased whole human blood and cryopreserved isolated human peripheral blood lymphocytes were evaluated for use in the in vitro chromosomal aberration assay. Initial testing was conducted in whole human blood with 5 donors and a long period of time to assess storage limitations. Cultures where propagated in triplicate and stimulated with phytohemagglutinin-M (PHA-M) to induce cellular division. The cultures were exposed to dimethyl sulfoxide (DMSO, 1%) at 48 hours post propagation, for a total of 4-hours with 59-activation and 22-hours without 59-activation. The cultures were harvested 22-hours post treatment and evaluated for mitotic index. Follow-up testing was conducted in whole human blood with 4 donors at 2, 5, and 7-days post-draw to assess shorter storage time and reproducibility of stimulation post storage. In addition, positive control responses were evaluated during the follow-up testing. Cultures were propagated in duplicate and stimulated with PHA-M. At 48-hours post propagation, the cultures were exposed to DMSO and cyclophosphamide or mitomycin-C. The cultures were harvested 22-hours post treatment and evaluated for mitotic index and cytogenetically analyzed for structural and numerical aberrations. Additional testing was conducted to assess the propagation capabilities of cryopreserved isolated human peripheral blood lymphocytes. The isolated lymphocytes were thawed and propagated in duplicate with PHA-M. At 48-hours post propagation, the cultures were exposed to DMSO and cyclophosphamide or mitomycin interaction of Aromatic Amines Related to the Occurrence of Bladder Cancer among the Exposed Workers

An Extended Evaluation of Commercially Purchased Blood and Cryopreserved Isolated Human Peripheral Blood Lymphocytes for Use in the In Vitro Chromosomal Aberration Assay

Integrated In Vivo Genotoxicity Testing to Follow-Up the Positive Ames Test of a Meptyldinocap Metabolite

Flow Cytometric Analysis of the HPBL In Vitro Micronucleus Assay—Proof of Concept

Integrated In Vitro Chromosomal Aberration Test to Meptyldinocap Metabolite
performed at two dose levels which equate to LOAEL and NOAEL for meptyldinocap. In this study, male and female Cr:CD1 mice (n=6) were fed diets supplemented with 0, 200 or 750 ppm X12335709 for 28 days. Blood samples were collected 7 days prior to the first dose for pre-exposure and on day 28 for post-exposure for gene mutation (CD59 marker for Pig-a) and MNT evaluations. The positive controls in this study were 20 mg/kg N-Nitroso-Niethylurea (days 1-3) and 40 mg/kg 4-hydroxy-1,3-benzoquinone (days 1-5) for Pig-a and micronucleus tests, respectively. During the study, the color of the urine was similar to the color of X12335709, indicating that animals had systemic exposure to X12335709. Adverse effects were not observed at any dose level, and both the Pig-a assay and MNT results were negative for mutagenicity and clastogenicity, respectively. The negative Ames assay, in vivo transgenic Big Blue® mice via inhalation exposure as well as dominant lethal study. In a 2-year carcinogenicity study via dietary route, increased benign liver tumors were observed in high dose male F344 rats at 25 mg/kg bw/day. To further examine whether genotoxicity is an initial key event in the tumorigenicity of 1,3-D, a fully OECD 488 compliant study was designed to be consistent with the cancer bioassay including selection of species, sex, exposure regimen. Twenty-four F344 homozygous Big Blue® male rats (six/group) were exposed daily to 1,3-D via diet (treatment groups) or placebo mixed in diet, for 28 consecutive days, at target dose levels of 0, 12.5, 25 or 50 mg/kg bw/day and left untreated for an additional 3 days for fixation. Animals were necropsied and liver and kidneys were collected at day 31. Genomic DNA was isolated from liver and kidneys, lambda phage shuttle vectors were recovered and inserted into empty lambda bac- teriophage capsids, creating infectious phage. Phage were adsorbed onto permissive E. coli G1250 host cells and plated out for plaque formation for mutants only under selective temperature conditions (24 ± 0.5°C) and total plaque formation (both mutant and wild-type phage) under non-selective (37 ± 1.0°C) temperature conditions. The mutant frequency (MF) of the cII gene were then determined for liver and kidneys. The results show the mean MFs for liver are 32.2, 23.4, 21.5, 33.4 and for kidneys are 19.1, 22.4, 25.4, 19.8, respectively, with the highest MF for liver. No statistically significant differences between treatment groups and the placebo group in either tissue. The positive control, ENU significantly induced (>3-fold) MF for both tissues, indicating the utility of the test system to detect induced mutants following exposure to a known mutagen. In conclusion, 1,3-D is negative for causing a statistically significant increase in mutations at the cII gene in the liver and kidneys of male Big Blue® rats. This conclusion coupled with previous in vivo genotoxicity results further confirms that 1,3-D is not an in vivo genotoxicant.

1553 1,3-Dichloropropene In Vivo Mutation Study in Male Big Blue Transgenic F344 Rats via Dietary Treatment

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1,3-Dichloropropene (1,3-D) is a pre-planting soil fumigant injected into soil for effective management of nematodes. Genotoxicity of 1,3-D has been extensively tested across all endpoints via GLP studies. While the in vitro Ames test exhibits mixed results, all in vivo studies are uniformly negative. Those studies include in vivo transgenic Big Blue® mice via inhalation exposure as well as dominant lethal study. In a 2-year carcinogenicity study via dietary route, increased benign liver tumors were observed in high dose male F344 rats at 25 mg/kg bw/day. To further examine whether genotoxicity is an initial key event in the tumorigenicity of 1,3-D, a fully OECD 488 compliant study was designed to be consistent with the cancer bioassay including selection of species, sex, exposure regimen. Twenty-four F344 homozygous Big Blue® male rats (six/group) were exposed daily to 1,3-D via diet (treatment groups) or placebo mixed in diet, for 28 consecutive days, at target dose levels of 0, 12.5, 25 or 50 mg/kg bw/day and left untreated for an additional 3 days for fixation. Animals were necropsied and liver and kidneys were collected at day 31. Genomic DNA was isolated from liver and kidneys, lambda phage shuttle vectors were recovered and inserted into empty lambda bacteriophage capsids, creating infectious phage. Phage were adsorbed onto permissive E. coli G1250 host cells and plated out for plaque formation for mutants only under selective temperature conditions (24 ± 0.5°C) and total plaque formation (both mutant and wild-type phage) under non-selective (37 ± 1.0°C) temperature conditions. The mutant frequency (MF) of the cII gene were then determined for liver and kidneys. The results show the mean MFs for liver are 32.2, 23.4, 21.5, 33.4 and for kidneys are 19.1, 22.4, 25.4, 19.8, respectively, with the highest MF for liver. No statistically significant differences between treatment groups and the placebo group in either tissue. The positive control, ENU significantly induced (>3-fold) MF for both tissues, indicating the utility of the test system to detect induced mutants following exposure to a known mutagen. In conclusion, 1,3-D is negative for causing a statistically significant increase in mutations at the cII gene in the liver and kidneys of male Big Blue® rats. This conclusion coupled with previous in vivo genotoxicity results further confirms that 1,3-D is not an in vivo genotoxicant.

1564 Analysis of the Genotoxic Potency of Pyrrolizidine Alkaloids Using HepaRG Cells in Conjunction with the GammaH2AX Assay

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Pyrrolizidine alkaloids (PAs) are secondary metabolites which occur in a large number of plant species and can be found as contaminants in food and feed. Several chemical classes of PAs can be distinguished according to the type of necine base and the esterification mode (mono-esters, open chain or cyclic diesters). In laboratory animals, the main toxicities resulting from treatment with 1,2-unsatuated PAs are genotoxicity, carcinogenicity and hepatotoxicity. It has been demonstrated that PAs have to be metabolically converted in order to exert their toxicity. The mutagenic risks of PAs for humans cur-
3Rs Friendly Study Design Facilitates Combined Rat Liver Micronucleus, Blood Micronucleus, and Pig-a Assays: Proof-of-Concept with Ten Diverse Genotoxicants


Regulatory guidelines stress the value of assessing multiple tissues and the most appropriate endpoints when evaluating chemicals for in vivo genotoxic potential. Yet conducting several independent studies to consider multiple endpoints and/or tissue compartments is resource intensive, and conventional approaches for scoring genotoxicity endpoints are inefficient. To address these issues with current practices we attempted to i) devise a resource-sparing treatment and harvest schedule that is compatible with liver and blood micronucleus (MN) endpoints, as well as the Pig-a gene mutation assay, and ii) utilize flow cytometry-based methods to score each of these biomarkers. Experiments were performed with 25-week-old C57BL/6 mice. Three groups were exposed to three 3-day cycles of hydroxyurea, quinoline, benz(a)pyrene (BP), cyclophosphamide (CP), diethylnitrosamine (DEN), cisplatin (CisP), 2,6-dinitrotoluene, 1,2-dimethylhydrazine, temozolomide, or vinblastine. Nine agents were dosed weekly for five cycles. In vivo MN and Pig-a responses were observed. For example whereas BP induced a clear increase in Pig-a mutant cells and a modest induction of blood MN, CP slightly affected Pig-a mutation but caused a robust blood MN response. BP nor CP induced liver MN. DEN caused a subtle Pig-a response, no induction of blood MN, and a robust liver MN response. Only one genotoxic agent, CisP, caused increases across every endpoint. Collectively, these results reinforce the importance of evaluating chemicals' genotoxic potential in both hematopoietic and liver compartments. These data also suggest it is possible to markedly enhance the efficiency by which several genotoxicity endpoints and two tissues can be evaluated through the use of flow cytometric scoring in conjunction with a resource-sparing study design that provides better utilization of fewer animals relative to current practices.


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The in vitro MultiFlow DNA Damage Assay multiplexes γH2AX, p53, phospho-histone H3, and polypliodization biomarkers into a single flow cytometric analysis. The current report describes a sequential data analysis strategy based on data generated from exposure of human TK6 cells to a previously described 85 chemical training set and a new, more pharmaceutical-centric test set (n=40). In each case, exposure occurred for 24 hr over a range of concentrations, and cell aliquots were removed at 4 and 24 hr for analysis. The data analysis pipeline described herein begins with a machine learning ensemble in addition to a rubric that considers fold increases in biomarker responses. The intra-lab reproducibility was further demonstrated with additional data, and these analyses were found to group certain chemicals, especially aneugens, according to their molecular targets. Finally, a third tier utilizes benchmark dose analyses and MultiFlow biomarker responses to rank genotoxic potency. The relevance of these rankings is supported by the strong agreement found between benchmark dose values derived from MultiFlow biomarkers compared to those generated from parallel in vitro micronucleus analyses. Collectively, the results suggest that a tiered MultiFlow data analysis pipeline is capable of rapidly and effectively identifying genotoxic hazards while providing additional information that is useful for modern risk assessment—MoA, molecular targets, and potency.

Development of Real-Time Human Cell Analysis Method for Identification and Categorization of Genotoxic Chemicals


Genotoxicity is a significant health concern leading to cancer and birth defects. Current testing methods are limited in that they can only detect a subset of genotoxic agents and suffer from potential interferences of cytotoxicity. Therefore, for a thorough assessment of genotoxic activity, a battery of end-point assays is required. Using human cell lines with intact DNA damage response pathways, we developed a Real Time Cell Analysis (RTCA) method that continuously monitors impedance values reflecting integrated signals from cell number, attachment, and morphology, generating kinetic Time-Dependent Response Profiles (TCRPs) indicative of mechanism of action. Upon exposure to genotoxic agents, DNA repair pathways are activated, leading to physiological and morphological changes that are recapitulated in the kinetic profiles, generating three distinguishable TCRPs for aneugens, clastogens/mutagens, and non-genotoxic agents, whilst simultaneously capturing cytotoxicity. An algorithm for TCRP analysis was developed to classify these sub-groups and a 50 compound screening library was formed composed of 33 genotoxic compounds working through different mechanisms, and 17 non-genotoxic compounds from OECD reference compounds list. Sensitivity and specificity of the TCRP method were tested with the Pig-a assay, with 32 out of 33 genotoxic positive agents identified, and 16 out of 17 negative agents tested as non-genotoxic. In addition, when combined with micronucleus (MN) flow assay, the workflow can further distinguish clastogens from mutagens. To our knowledge, this is the first testing method that can identify and classify multiple categories of DNA reactive chemicals, including clastogens/mutagens and aneugens. This model, when validated, can be used as a first tier high-throughput quantitative screening system for genotoxic agents.

Development of a 3D Genotoxicity Testing Battery Using Reconstructed Skin Models


The European regulation prohibits the use of animals in genotoxicity testing for ingredients used in cosmetics. Irreversible events at the chromosomal level are commonly evaluated by either scoring micronuclei or chromosome aberrations using cell-based assays, when assessing genotoxicity. These assays in 2D suffer from a poor specificity, and this is exacerbated in chemicals with skin as first site-of-contact due to the irrelevance of dermal exposure, lack of normal cell cycle control and intercellular microenvironment, as well as comparable metabolic capacity to native human skin. Reconstructed 3D human skin tissues overexpressing limitations of 2D in vitro assays, were therefore chosen in this study to build up a testing battery, enabling prediction of genotoxicity for chemicals. The current study was conducted on two commonly used in vitro genotoxicity assays using reconstructed skin models, an epidermis model EpiskinTM was used for micronucleus assay and comet assay was developed with a full thickness skin model. The EpiskinTM Micronucleus Assay was developed, and a formal multi-center validation study was conducted in China with two authority laboratories. The first phase was conducted with six chemicals selected by Cosmetic Europe Genotoxicity Taskforce: Phenantherene, Mannitol, D-limonene, 5-Fluorouracile, Vinblastine and ENU. Results showed correct predictive capacity in a 100% inter-laboratory test. To further demonstrate the characterization of EpiskinTM was conducted. Both gene expression and enzymatic activity analysis showed comparable metabolic capacity of EpiskinTM to human native skin and confirmed the correct classification of this assay for positive compounds requiring metabolic capacity (exc. 2-AAF). For comet assay, the protocol was firstly set up with two genotoxins (ANQO and MMS). A stable baseline level was established to extrapolate positive responses. The intra-lab reproducibility was further demonstrated with addi-
tional four genotoxic carcinogens at non-cytotoxic range of concentrations and the results showed good concordance with in vivo data. Altogether, these results indicate that genotoxicity assays using reconstructed skin models offer promising results for the safety assessment of chemicals with a dermal route of exposure, the availability and validity of these assays are expected to contribute to the in vitro genotoxicity testing strategy, the 3D genotoxicity testing battery could be used as ‘2nd tier’ assays to follow up on positive results from standard in vitro assays.

### 1571 Extension of the ToxTracker Reporter Assay for Classification of Compounds with an Clastogenic or Aneugenic Mode of Action


The distinction between a clastogenic or aneugenic mode of action is important for safety considerations. For clastogenic compounds that are directly DNA reactive, there is likely to be no safe level of exposure, while for aneugens that do not directly react with DNA, there might be a threshold below which there is no adverse effect. ToxTracker is a mammalian stem cell-based reporter assay that detects the activation of specific cellular signalling pathways upon exposure to compounds (Hendriks et al, Tox Sci 2016). ToxTracker contains six different GFP-tagged reporters that allow the discrimination between the induction of DNA damage, oxidative stress and protein damage in a single test. To assess whether ToxTracker can provide further insight into the mode of action of genotoxic compounds, we analysed various clastogenic as well as aneugenic compounds. The clastogens cisplatin and etoposide activate both markers for DNA double strand breaks (Rtkn) and replication stress (Bsc1). In contrast, aneugenic compounds that interfere with microtubuli, such as VCR, were defective in MMS-associated sister chromatid exchange. One hypothesis is that Shu1 suppresses the Srs2 helicase that promotes potential recombinogenic lesions into a mutagenic pathway. To test this hypothesis, we made csm2 srs2 double mutants. The mutants exhibited slow growth, like the csm2 mutant, but were still deficient in DNA damage-induced recombination. These studies have led to the hypothesis that the SHU complex participates in a pathway of mutation avoidance by directly participating in assembly of a Rad51 complex. Future studies will investigate whether the SHU complex suppresses mutagenicity after exposure to multiple genotoxins. These studies will thus elucidate the mechanisms that suppress carcinogen-associated mutation.

### 1572 Adaptation of the ToxTracker Reporter Assay for the Genetic Toxicology Assessment of Petroleum Products

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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, 18 petroleum substances (PS, highly- and poorly refined, with various levels of 3-7 ring PAHs) were tested in the ToxTracker reporter assay. PS are a panel of six validated mouse embryonic stem cell lines each containing a GFP reporter to detect induction of DNA damage, oxidative stress and protein damage in a single test. In parallel, all 18 petroleum extracts were also tested in the Modified Ames mutation assay. All tested highly refined base oils were classified as non-genotoxic in ToxTracker and non-mutagenic in the ModAmes test. Three petroleum distillate aromatic extracts (DAE) and four heavy fuel oil (HFO) extracts with higher levels of 3-7 ring PAHs, activated the genotoxicity reporters in ToxTracker and induced mutations in the ModAmes test. The poorly refined petroleum extracts also triggered oxidative stress and protein damage responses, in line with the (in vivo) toxicology of these extracts. Overall, results observed with ToxTracker correlated with those obtained from the established ModAmes assay. However, two technical issues related to the PAH content of PS were identified which are currently being further investigated: Firstly, PAHs are known to be autofluorescent, which could interfere with analysis of the GFP reporters. Therefore, activation of the six ToxTracker biomarker genes by the PS with high PAH levels was also confirmed by quantitative RT-PCR. Secondly, 3-7 ring PAHs generally require metabolic activation to become genotoxic. We evaluated the metabolic capacity of the ToxTracker cells by testing various PAHs with a defined battery of aprotic extracts from a S9 metabolic system. Furthermore, the S9 protocol form the ModAmes test was adopted in ToxTracker. Based on this, optimization of the ToxTracker protocol to address the specific complexities for (petroleum) UV/CB substances around autofluorescence and application of the S9 metabolizing system will improve the adequate genotoxicity assessment of PS.

### 1573 Genetic Control of Mutation Avoidance after Exposure to Carcinogens

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Aflatoxin (AFB1) is the most potent hepatocarcinogen known. The signature p53 mutation, p53-249<sup>th</sup>, found in AFB1-associated liver cancer suggests that AFB1 is a strong carcinogen because it is a mutagen. However, there is variability in the human response to AFB1 and polymorphisms in some DNA repair genes that participate in double-end break repair, such as XRCC1 and XRCC3, are risk factors for liver cancer. To understand genetic factors that suppress AFB1-associated mutagenicity we performed a genome profile for aflatoxin resistance in budding yeast, since many DNA repair and metabolism genes are orthologous to humans. To achieve this the human CYP1A2 was introduced into the deletion library to activate AFB1. Prominent among 86 genes that confer AFB1 resistance, are genes involved in error-free template switch mechanisms. These include genes in the SHU complex, consisting of CSMD2, PSY3, SHU1, and SHU2 that suppress methyl methanesulfonate (MMS)-associated mutations at the CA1 locus. Epistasis analysis revealed that CSMD2 and RAD51 are in the same epistasis group while CSMD2 and RAD4, which participate in nucleotide excision repair, are in different epistasis groups for AFB1 resistance. We observed that csmd2 mutants exhibit higher frequencies of AFB1-associated mutations but lower frequencies of AFB1-associated error-free template switching. Additional studies showed that csmd2, psy3, shu1, and shu2 were defective in MMS-associated sister chromatid exchange. One hypothesis is that Shu1 suppresses the Srs2 helicase that promotes potential recombinogenic lesions into a mutagenic pathway. To test this hypothesis, we made csm2 srs2 double mutants. The mutants exhibited slow growth, like the csm2 mutant, but were still deficient in DNA damage-induced recombination. These studies have led to the hypothesis that the SHU complex participates in a pathway of mutation avoidance by directly participating in assembly of a Rad51 complex. Future studies will investigate whether the SHU complex suppresses mutagenicity after exposure to multiple genotoxins. These studies will thus elucidate the mechanisms that suppress carcinogen-associated mutation.

### 1574 Investigation of the DNA Damage Response in Chemoresistant versus Chemosensitive Ovarian Cancer Cells

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Ovarian cancer is the fifth leading cause of cancer related deaths in women. The majority of patients are diagnosed at an advanced stage of the disease and many will experience disease recurrence. Several platinum compounds, including cisplatin, are currently used as anticancer drugs in the treatment of ovarian cancer. However, ovarian cancer patients often develop chemoresistance to current platinum/taxane chemotherapy regimens upon recurrence and these therapies tend to be the most effective at the time of diagnosis. Resistance in ovarian cancers. Modified comet assay was used to measure inter-strand cross-link (ICL) formation and repair, together with cellular base and nucleotide excision repair in chemosensitive (SKOV-3) and chemosensitive (OCI-P5x and A2780) ovarian cancer cells. Although the peak of cisplatin-induced cross-link formation for all three cell lines was at 12 h, and confirmed by ICP-MS, we noted a significant attenuation of ICL formation in the chemoresistant versus the chemosensitive cell lines. We also observed that for A2780 and OCI-P5x cells, ICL formation was higher in the chemoresistant cells than in the chemosensitive cells. The results indicate that the chemoresistant cells have a higher capacity to repair ICLs, suggesting that the chemoresistant cells are more able to repair DNA damage and are therefore better able to withstand the DNA damage induced by cisplatin. The results suggest that the chemoresistant cells are more able to repair DNA damage and are therefore better able to withstand the DNA damage induced by cisplatin.
considerably more resistant to damage formation, at all doses of cisplatin. These data provide a basis for further studies to improve our understanding of the differential DNA damage response associated with chemoresistant and chemoresistant ovarian cells. Elucidating the mechanisms of chemoresistance may ultimately lead to novel therapeutic interventions that may resemitize or prevent the development of chemoresistance.

**1575 Mutagenicity Study of a New 2-Phenylbenzotriazole, a Derivative of the Water Contaminant C.I. Disperse Violet Azo Dye**


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2-phenylbenzotriazoles (PBTA) are formed during the reduction reaction of dinitrophenyl azo dyes during the textile dyeing process. Some PBTA have already been described and found in the aquatic environment. The dinitrophenyl azo dye C.I. Disperse Violet 93 has been found in rivers in Brazil but their corresponding PBTA has never been described. This work is part of a project where a new PBTA derived from the C.I. Disperse Violet 93 was synthesized and characterized as N-(2-(6-aminio-4-bromo-2H-benz[d][1,2,3]triazol-2-yl)-5-(diethylamino)phenyl)acetamide. The objective of this study was to study its mutagenic activity using the Salmonella/microsome assay. We used the miniaturized protocol Microplate Agar (MPA) in the presence and absence of metabolic activation performed by rat liver S9 with 4 strains of Salmonella: TA98, TA100, TA102, and YG97a. We included two additional strains: the YG1108, a derivative of TA1535, which is highly sensitive to alkylating compounds, and the YG1041, a derivative of TA98, which is highly sensitive to compounds containing nitro/amino groups because of the increased expression levels of nitroreductase and O-acetyltransferase enzymes. The compound was tested diluted in dimethyl sulfoxide at limit of solubility, 0.83 µg/ul. Positive results were found for TA98, TA100, YG97a and YG1041 in the presence of S9. YG1041 was by far the most sensitive strain. Therefore N-(2-(6-aminio-4-bromo-2H-benz[d][1,2,3]triazol-2-yl)-5-(diethylamino)phenyl)acetamide has the ability to cause frameshift and base pair mutations in the Salmonella test. It is likely that the CYP enzymes provided by S9 are oxidizing the amino group to a hydroxylamine, which is further acetylated by the OAT enzymes, highly expressed in the YG1041. This would explain the high response of this compound to this strain in the presence of S9. Experiments are under way to verify its ability to cause DNA damage in vivo using comet assay in aquatic animals.

**1576 Ultra-Rare In Vitro Mutations Are a Sensitive and Specific Biomarker of Endogenous and Environmental Genotoxic Exposure**


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Somatic mutation accumulation is driven by endogenous and environmental processes. These processes imprint subtle, but characteristic patterns on the genomes of cells, and tell the story of life-integrated genotoxic exposure. Trinucleotide mutation signatures offer a rich target for exploitation as a biomarker for disease as they are often the result of frameshifts in introns that are modulated by exposure dynamics, tissue development, and lifestyle choices. However, detection in this setting remains challenging. The COSMIC trinucleotide mutation signatures are curated from the genomes of thousands of human tumors. The etiologies of these signatures have been studied in a wide range of tumors. We hypothesize that the presence of these signatures in healthy tissue during the long, and potentially informative period of cancer risk, prior to detectable tumor formation. Standard techniques for de novo mutation detection rely on clonal amplification of progenitor cells, for example, when researchers survey passenger mutations in a tumor, or when exposed cells in culture are grown out into easily assayable clones. These methods are fraught with confounders, such as bottlenecks and selective pressures, which alter or obscure the founder mutations. Duplex Sequencing is a tag-based DNA sequencing error correction method that enables detection of rare mutations at mutation frequencies of normal human tissues (~10e-8). Duplex Sequencing can be applied to the DNA of any tissue, of any organism, at any time, without the need for tumor formation or ex vivo cloning. To benchmark Duplex Sequencing detection of treatment-specific signatures in vivo, we assayed 5 tissues from mutagen treated mice before the any evidence of neoplastic disease. We observe mutation signatures corresponding to the context-specific mode of action of the mutagen with high correlations to previously published signatures from human and mouse tumor samples. Remarkably, these signatures are also present within the blood of the mice, suggesting the potential for using blood as the substrate for a non-invasive biomarker test of mutagenic exposure in model organisms and humans. This work highlights opportunity for increased understanding of carcinogenesis with Duplex Sequencing, which will enable the use of mutagenic signatures as an early biomarker of genomic insults, and of novel methods to mitigate cancer risk.

**1577 Naringenin Attenuates Genotoxicity Mediated by Oxaliplatin in Mice**


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Naringenin (NG) is a naturally occurring bioflavonoid that possess anti-oxidant and protective effects against DNA damage. Oxaliplatin (OX) is a third-generation platinum based anti-cancer drug used to treat various human malignancies, even though it mediates genotoxic effects in normal cells. The aim of this study is to examine the protective effects of NG on OX-induced DNA damage in mice. A total of 50 male mice (BALB/c) were randomly divided into five groups (10/group). Group I served as a control and was orally administrated 20mg/kg of NG. While group IV and V were orally administrated 20 and 40 mg/kg NG (NG20 and NG40), respectively, for ten consecutive days. On the tenth day of the experiment, animals in groups III, IV, and V were given a single intraperitoneal injection of OX (7 mg/kg) to induce genotoxicity. After 24 hr of OX treatment, mice were sacrificed. Measurements of GSH, GSSG and 8-OHdG levels and chromosome aberration analysis were carried out to evaluate DNA damage. GSSG and 8-OHdG levels were significantly induced by OX treatment by 1.24±0.21 nM/106 cells and 2.91±0.18 ng/L, respectively, compared to the control groups, while NG20 and NG40 treatments significantly reduced the induction of GSSG and 8-OHdG levels mediated by OX treatment to 0.96±0.16 nM/106 cells, 0.84±0.16 nM/106 cells, 2.31±0.43 ng/L and 1.81±0.39 ng/L, respectively. Both NG doses significantly counteracted the reduction of GSH mediated by OX treatment (2.9±0.3 nM/106 cells) by 4.1±0.4 and 5.2±0.4 nM/106 cells, respectively. Chromosomal aberration test showed a significantly higher proportion of chromosomal aberration at 32.33±2.97% mediated by OX treatment compared to the control group at 8.44±1.01%, while NG20 and NG40 significantly reduced the frequency of chromosomal aberration to 20.67±28.88% and 15.39±2.75%, respectively, in dose dependent manner. In conclusion, these findings suggest that NG significantly reduced the DNA damage induced by Oxaliplatin possibly due to its anti-oxidant effect and propose it as a potential chemoprotective agent against genotoxicity associated with chemotherapy.

**1578 Comparative Cardiovascular Parameters in Bama and Göttingen Minipigs**


The minipig has been accepted and recognized as a suitable alternate non-rodent species in both biomedical research and new drug development. Investigations demonstrated the advantages of miniature pig when used as the model for evaluating drug candidate: it has similar cardiac anatomy, metabolism and electrophysiology when compared with human. The Göttingen minipig is well known and routinely used for regulatory cardiovascular safety pharmacology study. Meanwhile, the Chinese Bama minipig, one of the miniature pig breeds, is increasingly used in China due to the features of genetically stable, highly inbred and cost and availability. The objective of this study was to compare the electrocardiographic parameters between Göttingen and Bama minipigs. In addition, we also compared the electrocardiographic parameters collected by both telemetry and non-telemetry. The study results indicated no breed difference or sex difference in the heart rates, RR, PR, QRS and QT intervals between Göttingen and Bama minipigs. The non-telemetry heart rate was generally higher than that collected with telemetry method. Diurnal rhythm was noted in heart rate and QT/QTc interval for Bama miniature pigs. In conclusion, the electrocardiographic parameters are comparable between the Göttingen and Bama minipigs, and also the strain of Bama minipig is considered suitable for cardiovascular safety pharmacology study.
1579 Immunology Historical Data of 2-4 Years Cynomolgus Monkey (Macaca fascicularis) from Hainan, China


The Cynomolgus monkey (Macaca fascicularis) is a well-known non-human primate species commonly used in non-clinical research especially in the toxicology studies of the biopharmaceutics. Since the historical pretest data is limited for immunology parameters in the Cynomolgus monkey, the data collection and evaluation was conducted from 7 toxicity studies in Wuxi AppTec Suzhou Facility from 2015 to 2018. Animals were 2-4 years Cynomolgus monkeys and the supplier is Hainan Jingang Biotech Co., Ltd. Pretest and control animal data from completed and ongoing toxicology studies were used to summarize the immunological historical data of three aspects: B and T Lymphocyte phenotyping (up to 338 monkeys, 169/sex), cytokine analysis (up to 310 monkeys, 155/sex) and immunoglobulin and complement analysis (up to 136 monkeys, 68/sex). At initiation of dosing, monkeys body weights ranged from 2.3 to 4.0 kg for males and 2.1 to 3.6 kg for females. Blood samples for B and T Lymphocyte phenotyping, cytokine, immunoglobulin and complement analysis were obtained from a cephalic or a femoral vein. Blood collected for B and T Lymphocyte analysis were Anticoagulant with K2EDTA. Serum for cytokine, immunoglobulin and complement analysis were obtained by standard refrigeration. B and T Lymphocyte phenotyping was conducted using a qualified flow cytometry method (Becton-Dickinson FACSCanto II Flowcytometry System). Cytokine analysis was conducted using a qualified Cytometric Bead Array method based on flow cytometry (Becton-Dickinson FACSCanto II Flowcytometry System). Immunoglobulin and complement concentrations in the serum were measured using a validated immunoturbidimetric method based on biochemistry analyzer (Hitachi 7180 Biochemistry Analyzer). Results of the B and T Lymphocyte (Total T cells, Helper T cells, Cytotoxic T cells, B cells), immunoglobulin and complement (C3c, C4, IgG, IgA and IgM), and cytokines (IL-2, IL-4, IL-5, IL-6, TNF-α and IFN-γ) were showed as mean, standard deviation (SD) and Coefficient of Variance (CV) to demonstrate the historical data and the variation between studies and individual animals.

1580 Potential Endocrine-Disrupting Effects of Graphene Oxide Nanomaterial on Japanese Medaka Fish

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Due to the unique physico-chemical properties, graphene oxide (GO) has shown great potential for biomedical, energy and electronic applications. As a result, release of GO into the ecosystem especially into the aquatic environment is inevitable; however, the potential risks on aquatic life are almost unknown. We have evaluated the toxic potential of GO on Japanese medaka fish (Oryzias latipes) reproduction. GO was synthesized by modified Hummer’s method in the laboratory and was characterized by Fourier transform infrared (FTIR), field emission scanning electron microscopy (FIB-SEM), transmission electron microscopy (TEM), AFM (Atomic Force Microscopy) and Zetasizer Nano ZS. The reproductively active adult medaka were injected (25 µg/g with GO (274.1±46 nm in diameter) and allowed for normal breeding for three weeks; the eggs laid were evaluated for developmental abnormalities including hatching. Moreover, histological studies were performed on the gonads (testis and ovary) of the parents three weeks post injection. It was observed that even though GO administration to adult fish was unable to induce any significant effects on fecundity and developmental abnormalities in hatched larvae, the testicular degeneration as evidenced by germ cell synctiata in males and significant follicular atresia in ovary of females indicated a potential endocrine disrupting effect. As GO is a known inducer of oxidative stress, testicular degeneration in males and disruption of folliculogenesis in females are probably the result of oxidative damage.

1581 Attenuation of Lung Inflammation and Emphysema-Like Changes following Cigarette Smoking Cessation or Switching to Aerosol Inhalation from an NTV


Mainstream cigarette smoke (CMS) is one of the risk factors for development and progression of respiratory diseases, such as chronic obstructive pulmonary disease (COPD). Novel tobacco vapor product (NTV) is a product with low-temperature heating technology. Previous study found that the vapor from NTV showed distinctively lower yields of potentially harmful constituents than CMS. We used a murine model of COPD to investigate the effects of chronic exposure to NTV aerosol, compared with CMS from 3R4F reference cigarettes (3R4F); and the effects of cessation and switching to NTV after 2 months of CMS exposure. Female C57BL/6 mice were exposed to 3R4F CMS (700 µg total particulate matter (TPM)/L), filtered air (FA) or NTV aerosol (700 µg TPM/L) for up to 6 months. In both groups, after 2 months of CMS exposure, mice were exposed to FA (cessation) or NTV (switching) for 4 months. All groups underwent intermittent exposure (i.e., 4-hour daily exposure arranged as two 2-hour periods with a 30-minute break of FA exposure). After completion of exposure, mice were examined for emphysema-related changes as well as several toxicological endpoints. Two-month CMS 3R4F CMS exposure led to upregulated inflammation-related gene expression in lungs and increased proteolytic enzyme activities in bronchoalveolar lavage fluid, associated with alveolar destruction, and perturbed lung function, indicating emphysema-like changes; whereas exposure to NTV aerosol did not induce these effects at any time points. The effect of NTV aerosol on biological processes was very similar to that of FA. Both cessation and switching attenuated the effect of 3R4F to levels similar to FA. These data clearly show that NTV aerosol has little impact on biological processes (inflammatory responses, emphysema-like changes and impaired alveolar damage), compared with 3R4F CMS in a murine model of COPD. Furthermore, it suggests that effects of switching to NTV on these processes might be similar to those of cessation.

1582 Positive Control Study of Urethane and MNU in TgRasH2 Mice

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The TgRasH2 [CByB6F1-Tg(Hras)2Jic, (TgRasH2)-hemizygous (+/-)] mouse is the genetic model for detecting carcinogenic potential of genotoxic and non-genotoxic test articles in 26-week assays and has been found a suitable model by the US FDA. A positive control group is recommended for inclusion. This study compared the efficacy of urethane and N-methyl-α-nitrosourea (MNU) as positive control carcinogenic substances for validation of the TgRasH2 mouse carcinogenicity assay. Ten mice/sex/group were administered urethane (1000 mg/kg) or saline by intraperitoneal injection on Days 1, 3, and 5, or MNU (75 mg/kg) on Day 1. Animals administered MNU or urethane were sacrificed after 16 weeks of observation and saline control animals after 26 weeks. One control male and another female, and two females administered urethane, and one male and three females administered MNU were found dead or sacrificed moribund between Weeks 13 and scheduled sacrifice, all attributed to generalized debilitation resulting from tumors. Neoplasia in the saline controls was rare. The control male sacrificed moribund had a gastric squamous cell carcinoma, a bronchiolo-alveolar adenocarcinoma, a hepatocellular adenoma, and a Harderian gland adenoma. A Harderian gland adenoma was also present in one control male sacrificed at Week 26. All animals administered urethane experienced transient decreased activity, unsteady gait, and impaired righting after administration which was related to the anesthetic effect of urethane. Skeletal muscle changes were present in all animals of all groups, consistent with a degenerative myopathy described in TgRasH2 mice. All animals treated with urethane had proliferative lesions in the lung (bronchiolo-alveolar hyperplasia, adenoma, and carcinoma) and spleen (hemangiosarcoma). All animals treated with MNU had atrophy of the stomach (gastritis) and squamous cell carcinoma as well as multi-centric lymphoma, consistently affecting the thymus. Results indicate that examining lungs and spleen at Week 16 from animals administered urethane, or examining stomach and thymus at Week 16 from animals administered MNU is sufficient to reliably demonstrate carcinogenicity in the positive control group.

1583 Biochemical Studies on Toxicity of Yoyo Bitters (A Polyherbal Preparation) on Kidney and Liver Functions of Wister Rats


Use of herbal preparations in the treatment of diseases especially in Asia and Africa is becoming increasingly popular. However, there is dearth of scientific evidence on the toxic effects and pharmacological claims of most of them. Toxicity of a popular Nigerian herbal preparation (Yoyo Bitters) on the kidney and liver of Wister rats was investigated over a period of forty-one days in Wister rats (cases) and forty others (controls) all weighing averagely 120gm. Cases were fed with normal rat chow and given the herbal preparation in a single dose of 200 mg/kg bwt. Control rats were given normal rat chow only. The NAS, alkaline phosphatase, ALT and AST were significantly higher in cases than in controls. The highest increase was observed in alkaline phosphatase (51% increase) followed by ALT (24% increase), AST (19% increase) and NAS (10% increase). The Urea, total protein, albumin, total bilirubin and creatinine had significantly higher values in cases than in controls. The highest increase was observed in creatinine (13% increase) followed by albumin (10% increase), total protein (10% increase) and total bilirubin (6% increase). The highest decrease was observed in urea (24% decrease) followed by total protein (14% decrease) and albumin (11% decrease). The ALT and AST activities were higher in male rats than in female rats while NAS, alkaline phosphatase and total bilirubin activities were higher in female rats than in male rats. In conclusion, the results showed that Yoyo Bitters has significant hepatorenal effects on Wister rats.
ALP, alanine amino transaminase (ALT) and Aspartate amino transaminase (AST) were also estimated as liver function indices spectrophotometrically in cases and controls at specific intervals of the study. Mean values ranged from 5.50±0.70 - 8.30±0.46; 7.50±1.80 - 24.90±10.00; 0.70±0.20 - 0.85±0.01 and 1.40±0.80 - 6.50±2.20 for plasma calcium, inorganic phosphorus, creatinine and uric acid in cases respectively. Values for inorganic phosphorus, creatinine and uric acid were significantly different. Mean liver function values ranged from 1.40±0.06 - 0.60±0.20 and 0.64±0.10 in cases against 0.92±0.20 and 0.27±0.20 in controls for total and conjugated bilirubin respectively. Mean enzyme activities ranged from 201±28 - 88±35, 172±100 - 52±23 and 426±71 - 146±90 in cases as against 340±51, 158±61 and 347±136 in controls for ALT, AST and respectively. A scattered plot and correlation analysis of the data showed a flat curve and no significant correlation in the levels of these parameters except in the uric acid which showed a significant proportional increase over the 42-day period of the experiment. Administration of this herbal preparation did not show a significant alteration in the function of the liver and kidneys and may not be an indicator for the changes in both glomerular and tubular functions of the kidney in the experimental animals. Observed hyperuricemia may be more of antioxidant properties of the preparation.

**1584 Development of a Nonchemical Stress Model for Environmental Studies: Effect of Maternal High-Fat Diet on Postnatal Cardiac Stem/Progenitor Cells**


Stem cell biology research has demonstrated a critical role of cardiac stem/progenitor cells (CSCs/CPCs) in heart homeostasis, injury repair, function, and disease susceptibility at all life stages. The impact of environmental toxicants and chemical stressors influences the effects which chemical and/or pollutant exposures may have on postnatal CSCs/CPCs is unknown. To address this uncertainty, research was conducted to examine the impact which maternal diet has on CSCs/CPCs levels in their offspring with intention of employing this model to study the effects of these AEDs on low frequency epileptiform spike activity. Following SE, individuals may develop spontaneous recurrent seizures (SRS) or epilepsy that often leads to severe neurological deficits and lower quality of life. Though dozens of US FDA-approved antiepileptic drugs (AEDs) are on the market, it is unknown which, if any, would be effective against NA-induced SRS. We designed this experiment to test five AEDs (levetiracetam, phenobarbital, valproate, carbamazepine, and clonazepam) in telemetry-instrumented mice over a 30-day period following a sublethal, supraconvulsant soman (GD) challenge. Starting 24 hours after SE, mice were repeatedly dosed with an AED or vehicle for 14 days, followed by a 14- day washout period. Dosing regimens were based on published pharmacokinetic data. Levetiracetam had no apparent effect on SRS number, duration, or onset of SRS. Although phenobarbital-treated mice exhibited a delay in SRS onset compared to vehicle, mice developed phenobarbital resistance prior to the end of the treatment period. Data are still being acquired on the effects of valproate, carbamazepine, and clonazepam on SRS activity. Continued analysis will focus on the effects of these AEDs on low frequency epileptiform spike activity. Although we have not found a suitable AED candidate to repurpose for our SRS treatment, we have developed a reliable animal model of treatment resistant epilepsy. With this animal model, AED candidates could be better characterized and more appropriately prescribed for epilepsy patients.

**1585 Increased Non-biliary Cholesterol Excretion during Alpha-Naphthylisothiocyanate (ANIT)-Induced Cholestasis in Mice**

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Excessive cholesterol accumulation in the body promotes the development of atherosclerotic cardiovascular disease. Bile has been considered the main route of elimination of cholesterol from the body. However, recent evidence suggests that the small intestine plays a role in excreting serum cholesterol through a process known as transintestinal cholesterol excretion (TICE). During cholestasis, increased serum bile acids are excreted into urine and cause numerous adaptive changes in bile acid homeostatic gene expression in kidney as well as liver. Accordingly, it is hypothesized that non-biliary cholesterol excretion and changes in the expression of cholesterol homeostatic genes occur in extrahepatic organs during cholestasis. To test this hypothesis, eight-week-old male C57BL/6 mice were administered alpha-naphthylisothiocyanate (ANIT) (75 mg/kg po) and 72 hours later liver, kidney, duodenum, jejunum, ileum, blood, and fecal samples were collected. Total bilirubin, ALP, and ALT were elevated in serum of ANIT-treated mice. Bile acid and cholesterol concentrations in serum and liver were increased after ANIT. These data indicate that ANIT induces both cholestasis and hypercholesterolemia. Furthermore, fecal and urinary cholesterol concentrations were also increased 72 hours after ANIT. In liver, ANIT did not alter Abcg5, Abcg8, Npc1, Ldlr, and Srb1 mRNA expression compared to control mice. In duodenum, ANIT tended to increase the cholestero uptake Ldlr mRNA expression. In jejunum, ANIT increased the mRNA of Srb1 as well as Ldlr, and decreased mRNA expression of Abca1 and Abcg5. In ileum, ANIT did not alter mRNA expression of cholesterol transporters and receptors. In kidney, ANIT tended to increase Srb1, Npc111, and Abcg8 mRNAs. These data indicate that in addition of ANIT being cholesstatic it is also hypercholesterolemic. The induction of intestinal Ldlr and Srb1 and trends of induced renal Srb1 and Abcg5 may be adaptive responses to ANIT-induced cholestatic liver injury and responsible for the increased fecal and urinary cholesrerol excretion, respectively.

**1586 Evaluation of Repurposed Antiepileptic Drugs to Treat Spontaneous Recurrent Seizures in Instrumented Male C57BL/6 Mice following a Sublethal Soman Exposure**

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Exposure to nerve agent (NA) can result in rapid onset of generalized status epilepticus (SE), which if uncontrolled can cause permanent brain damage and death. Many efforts have focused on ways to stop the initial seizure, but few have addressed the long-term complications and complications of exposure. Following SE, individuals may develop spontaneous recurrent seizures (SRS) or epilepsy that often leads to severe neurological deficits and lower quality of life. Through dozens of US FDA-approved antiepileptic drugs (AEDs) are on the market, it is unknown which, if any, would be effective against NA-induced SRS. We designed this experiment to test five AEDs (levetiracetam, phenobarbital, valproate, carbamazepine, and clonazepam) in telemetry-instrumented mice over a 30-day period following a sublethal, supraconvulsant soman (GD) challenge. Starting 24 hours after SE, mice were repeatedly dosed with an AED or vehicle for 14 days, followed by a 14- day washout period. Dosing regimens were based on published pharmacokinetic data. Levetiracetam had no apparent effect on SRS number, duration, or onset of SRS. Although phenobarbital-treated mice exhibited a delay in SRS onset compared to vehicle, mice developed phenobarbital resistance prior to the end of the treatment period. Data are still being acquired on the effects of valproate, carbamazepine, and clonazepam on SRS activity. Continued analysis will focus on the effects of these AEDs on low frequency epileptiform spike activity. Although we have not found a suitable AED candidate to repurpose for our SRS treatment, we have developed a reliable animal model of treatment resistant epilepsy. With this animal model, AED candidates could be better characterized and more appropriately prescribed for epilepsy patients.

**1587 Validating Zebrafish for Safety Drug Screening: A Comparison of Zebrafish and Rodent Toxicity Data**


In recent years, the zebrafish has become a valuable model. It is gaining popularity in the field of safety pharmacology due to its unique biological advantages. There is a gap between high-throughput in vitro studies and low-throughput in vivo studies during the drug discovery pipeline. The use of zebrafish in drug screening could fill that gap and become an important asset to assess toxicity and efficacy of novel drugs. Zebrafish has, from early developmental stages, fully functional organs from a physiological point of view and large transparent progeny, which develops externally and make them ideal for non-invasive approaches, such as in vivo imaging. Despite these advantages, the use of zebrafish is still scarce in current drug discovery pipelines and requires further validation to achieve a full adoption by the regulatory agencies. Zebrafish assays will only be useful if they can show high predictive power. Thus, we used our toxicological data (LC50) obtained in zebrafish embryos and have compared with LD50 values obtained from rodents (mice and rats), to evaluate the predictivity of the model. Besides, we performed drug bioavailability studies of the same compounds in zebrafish embryos to address the role of drug permeability in toxicity assessment. The aim of this study is to examine how the covariance of zebrafish and rodent toxicity is influenced by factors such as compound type, absorption, metabolism and mechanism of toxicity. For this purpose, we have compared the effects of ten compounds administered in rodents (oral, intraperitoneal and intravenous) have been compared to our
drug incubation. For the compounds examined here, we have seen that drug incubation better correlates with LD50 values obtained via intravenous route, than with intraperitoneal or oral respectively. Furthermore, we have analysed if the log P (partition coefficient value could have any impact in the drug toxicity seen in the embryos. Our preliminary results suggest that even though there is no strong correlation between LC50 and log P, those compounds which are more hydrophilic (less lipophilic) and therefore more water-soluble tend to be less toxic than the ones which possess high log P values (more lipophilic, less hydrophilic, less water-soluble). Our results will allow placing the use of zebrafish-based drug screening in an even better position for biomedicine discovery purposes. More work is required, but we hope that this and other translational validation studies could help to promote the zebrafish as a potential alternative model to toxicology testing.

1588 Strength of Litter Effect and Litter Size Effect in Body Weight Data across Multiple Toxicity Studies in Harlan Sprague-Dawley Rats


In developmental and reproductive toxicology (DART) studies of toxic substances in rodents, characterization of dose effects can be confounded by clustering of responses by litter, which can occur due to shared genetics and shared maternal and lactation environments. Using an extensive National Toxicology Program (NTP) database, we present analyses of rodent fetal and pup body weights and their relation to litter size as the rat ages. The intra-class correlation (ICC), a measure of the strength of litter effect, is highest during the pre-weaning period, such that the effective sample size (ESS) is very close to the number of litters contributing pups. Over the first 100 postnatal days, ICC values decline yet stabilize at a value corresponding to an ESS that is closer to the number of litters than to the number of pups on study. Body weights show an inverse relationship with litter size during the pre-weaning period, providing evidence that competition for milk supply may be a key determinant of growth rate. When pup weights are adjusted for litter size, a litter effect is still evident during the pre-weaning period, indicating that siblings have more in common than just the fetus count in utero. These results show that the interpretation of toxicity studies should include appropriate statistical modeling of litter structure.

1589 Disease Sensitive and Resistant Mouse Strains Identified in a Mouse Aging Study

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Genetic variability is an important factor in age-related development of disease. The NIEHS/NTP has conducted a multistrain “Mouse Aging Study” to examine genetic diversity as a variable for development of non-neoplastic and neoplastic diseases (lesions). Ten mouse strains representing ~90% of genetic diversity in laboratory mice were selected for this study. Preliminary evaluations indicate that there is a strain dependent susceptibility for neoplasms of the liver, lung, uterus, ovary, mammary tissue, skeletal muscle, and lymphoid organs. The incidence of neoplastic lesions ranged from 10% - 87% in the 10 mouse strains aged for 2 years (corresponding to about 70 human years). The data from this study complements information in the Jackson Laboratory Mouse Phenome Database and the Mouse Tumor Biology Database. Current safety assessments based on only a few animal strains with limited genetic diversity do not fully capture the range of variations in disease susceptibility to environmental hazards and drugs. Data on background disease susceptibility from the Mouse Aging Study allows more appropriate selection of mouse strains for toxicity studies and enhances the ability to identify mouse models to characterize diseases associated with aging. This study can also contribute information that may be useful for identifying genetic variants that influence susceptibility or resistance to disease development.

1590 Optimized Methods for Irradiation and Chemical Myeloablation in NSG Mice Prior to Human Hematopoietic Stem Cell (Gene) Therapy

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NOD.Cg-Prkdcscid I2rgtm1Wjl/SzJ mice (NSG) remain the gold standard for cell mediated gene therapy using transduced human hematopoietic stem cells (HSCs). Nearly complete ablation of the bone marrow of NSG mice is critical for successful xenotransplantation of the transduced cells and can be evidenced by the identification of the transduced HSCs in the peripheral blood and tissues. In this study, bone marrow ablation was approached by two different methods following IACUC approval: one by exposure to gamma irradiation, the other by administration of busulfan, a clinically utilized myeloablating agent. Because mice susceptibility to irradiation varies between strains and with age, it is necessary to optimize the dose of irradiation to achieve nearly complete bone marrow ablation. For irradiation intensity optimization, male NSG mice of 9 to 12 weeks of age were exposed to irradiation intensities of 2.00, 2.25, 2.5, 2.75 or 3 Gys, and female mice of 9 to 12 weeks of age were exposed to 2.25 or 2.75 Gys under a gamma irradiator. Post-irradiation exposure, clinical signs and body weight were closely monitored for a period of 28 days. For the males, the LD\(_{50}\) was approximately 2.5 Gy with 4 out of 7 males surviving until Day 28. In females, 3 out of 4 females survived at 2.75 Gy and all survived at 2.25 Gy. Considered together, the data indicated that 2.25 Gy as optimal irradiation intensity in male NSG mice, and 2.5 Gy as optimal intensity in female NSG mice. Another approach for bone marrow ablation was by intravenous infusion of busulfan, a clinically used bone marrow ablating agent. Busulfan was administered intravenously at an optimal dose of 15 mg/kg/day in male and female NSG mice for 3 consecutive days approximately 24 hours apart; the last dose of busulfan was administered approximately 24 hours prior to the cell implantation. This dose of busulfan was well tolerated by NSG mice and nearly complete bone marrow ablation was evidenced by consistent cell engraftment in NSG mice. Thus, it can be concluded that busulfan administration provides a translatable approach for xenotransplantation.

1591 Establishment of a Small Nonhuman Primate Colony for Ethical Reuse of Animals in Preclinical Safety Studies, Including Small and Large Molecule Research


The intensified development of biologics has necessitated increased safety testing in Nonhuman Primates. Since biologic drugs are either substantially or fully humanized, the Nonhuman Primate (NHP) is often the most relevant model when predicting human safety. Another challenge posed by biologics concerns the development of antidrug antibodies (ADA) in response to drug exposure which typically precludes the re-use of an animal for safety assessment. All of this runs counter to our goal of minimizing the use of NHPs in preclinical development. To overcome these challenges a small non-terminal chronic testing NHP colony was established as a global platform to enable the re-use of NHPs across all therapeutic areas requiring chronic toxicity testing of both biologic and low molecular weight (LMW) compounds. In order to screen NHPs and determine their suitability/availability for reuse, a generic qualitative ELISA based ADA binding immunoassay was developed to identify ADA positive and negative samples from previously exposed animals. NHPs testing positive are no longer suitable for use in biologics studies but are made available for LMW studies. Animals testing negative can be put on study using an unrelated biologic to which they have not been previously exposed or undergo a drug specific ADA screen prior to being dosed with a previously tested compound. To date the NHP colony has been expanded to contain 55 animals. Out of the 39 cyno samples collected from the colony which directly relates to the number of animals put on large molecule studies approximately 6 samples have tested positive for ADAs. These results indicate that 33 (~80%) animals can be reused for other studies. The development of this innovative screening assay and the reuse of animals for chronic safety testing represents a potential reduction in the number of NHPs used in preclinical development.
Electrolyte Quantification in Aqueous and Vitreous Humor of Common Preclinical Species


Ocular health assessment supporting standard preclinical ocular examination has generated interest in ocular toxicology testing. Few studies have quantified aqueous and vitreous humor electrolyte contents across species. This study aimed to quantify electrolytes in aqueous and vitreous humor from four preclinical species. Aqueous and vitreous humors were collected from rabbits (n=7), dogs (n=9), monkeys (n=10) and minipigs (n=7). Vitreous and aqueous humors were analyzed by Cobas 6000<sup>®</sup> c (plasma/serum mode).

Electrolyte concentrations (mmol/L) in aqueous and vitreous humor were comparable between species. Vitreous electrolyte concentrations were: phosphorus (0.3±0.07 to 0.3±0.19); calcium (1.4±0.18 to 2.3±0.33); sodium (132.8±16.5 to 157.0±17.5); potassium (5.1±0.57 to 5.3±0.36); chloride (99.4±14.53 to 116.18±5.48). Aqueous electrolyte concentrations were: phosphorus (0.72±0.05 to 0.90±0.08); calcium (1.25±0.10 to 2.18±0.04); sodium (142.00±6.11 to 153.67±4.58); potassium (3.8±6.06 to 5.26±0.99); chloride (97.80±4.07 to 116.60±2.97). Humor concentrations of sodium, potassium and chloride were generally similar to species-specific serum levels; however, phosphorus and calcium concentrations were slightly below serum levels.

This study provided a baseline/reference of humor electrolytes. Electrolyte measurement from aqueous and vitreous humor could be a useful parameter in the assessment of ocular toxicity in toxicology studies. Electrolyte composition of these anatomical segments may also have implications relative to test compound chemistry after intra-ocular delivery.

A Simple In Vivo Model for Monitoring CYP3A Induction and Inhibition

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In vitro cytochrome P450 (CYP) assays are used to predict in vivo drug interactions caused by CYP inhibition or induction. Inhibitors are typically identified in biochemical assays and inducers in cultured cells such as primary hepatocytes. However, it is desirable to confirm the predictive implications of in vitro results in an animal model. As CYP3A enzymes are prominent drug metabolizers involved in drug interactions in humans and animals we set out to establish a simple model for monitoring CYP3A induction and inhibition in vivo. To that end we used 2-(4-diisopropoxymethyl)-4,5-dihydrothiazol-2-yl)benzod[<i>d</i>]thiazol-6-ol (luciferin isopropyl acetal) as a probe CYP3A substrate in live mice. This probe is converted by CYP3A enzymes in vitro to a D-luciferin ester that is detected by luminometry in a luciferase formulation. Reactions with luciferin isopropyl acetal are highly CYP3A selective in humans and rodents and it was also used for bioluminescent imaging of cyto3a activity in the livers of live mice expressing a luciferase transgene. From this background we reasoned that endogenous cyto3a activity in wt mice (e.g. in liver) would convert the probe and if there is significant renal elimination of the reaction product it would appear in urine. This would be detectable by luminometry when urine samples are mixed with a luciferase formulation. The initial rate of metabolite appearance would reflect the cyto3a conversion rate. A maximum tolerated dose study with FVB/N mice showed no signs of probe toxicity, indicating it is well tolerated after IP injection. FVB/N urine samples did produce luminescence after IP probe injection indicating the probe is converted to a luminogenic product that is eliminated in urine. Signals peaked in samples collected at about 1 hour post injection and declined thereafter. Pretreatment of the mice with the cyto3a inducers pregnenolone 16a contraceptive or dexamethasone enhanced AUCs from samples collected in the first 3 hours post probe-injection. In contrast the cyto3a inhibitor troelodamycin reduced AUCs. To control for urine concentration, luminescence from these samples was normalized to urinary creatinine levels. The appearance in urine of a luminogenic product of the cyto3a-selective probe and modulation of the initial elimination rate by cyto3a induction and inhibition is consistent with cyto3a-dependent probe metabolism. This supports the notion that luciferin isopropyl acetal can be used as an in vivo probe for predicting drug interactions due to cyto3a induction or inhibition.

Assessments of Sexual Maturity in Three Lineages of Miniature Swine

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Sexual maturity can have multiple definitions depending on the area of research being discussed. Medical reproduction can be defined by the peak of reproductive capabilities, however, in toxicology studies it can be defined as the presence of reproductive gametes. The main objective of this study was to determine the onset of sexual maturity in males and females of three lineages of miniature swine, namely, Hanford, Sinclair S-1, and Yucatan, from data gathered in toxicology studies. A second objective was to evaluate the predictability of clinical measurable variables in identifying the onset of sexual maturity in the same strains of miniature swine. For the purpose of this study, sexual maturity was defined as having spermatogonial and spermatozoa in the epididymis in the males or the presence of corpora lutea in the female from histological evaluation of the testes or ovaries. The retrospective evaluation of histological findings of control males and females of different ages assigned to toxicology studies were assessed for sexual maturity. We found that all females reached sexually maturity before 16-19 wks of age regardless of the lineage. Males had reached sexually maturity by 19 wks for Yucatans, and 22 wks for Sinclair S-1 and Hanford. Several other clinically measurable variables were assessed as well from satellite animals and compared to the histological findings in an effort to determine the predictability of each variable in predicting the onset of sexual maturity. In males, testicular weight and the presence of preputial sperm appeared to be good indicators of sexual maturity. However, in the female, many clinical measurable variables such as vulvar measurements, swelling and discharge were found to be unreliable. In addition, estrus behavior is not a good indicator in miniature swine. The first objective of this study was reached in that the sexual maturity in these three lineages of miniature swine is well defined. We have shown that there are many methods of assessing sexual maturity in miniature swine, however many of them have been shown to be unreliable, especially for the female. Developing new clinical methods for assessing sexual maturity will be critical as the miniature swine increases in popularity for translational biomedical research.

Miniature swine have many advantages over other species for biomedical research such as their anatomical and physiological similarities to humans. However, one of the disadvantages of the miniature swine for biomedical research such as pharmaceutical toxicology can be the size of the animal. With a larger size test animal, a larger amount of test material is required to complete the toxicology study thus increasing cost of the study. The objective of this study was to utilize genomic selection, phenotype and body weight, to reduce the total body mass in adult Sinclair S-1 miniature swine. Initially, a large baseline single nucleotide polymorphism (SNP) bank was established for each breeder of the Sinclair miniature swine colony. The SNPs were then used to calculate genomic estimated breeding values for body weight of the breeding animals. Finally, we developed a computer algorithm to manipulate a large dataset of phenotypic and genomic data to determine the optimal mating combinations to result in a reduction in adult body mass. Utilizing this genomic selection criteria, we have observed an 8% decline in body size per year in the Sinclair S-1 miniature swine. After 5 years of this genetic selection, the body weight reduction outcome of the 4th generation at 6 months of age, is a significant (p<0.05) reduction in body weight in both males (35.1%) and females (25.1%). Further concurrent studies are being conducted to standardize the clinical pathology, biometric and reproductive variables, as well as validation studies for compound kinetics, dermal irritation, and feeding regimen assessment. We have developed a genomic selection tool based on body mass to down size the Sinclair S-1 miniature swine over the course of the next several generations. As the pig becomes the accepted translational model for biomedical research, we believe that strategically downsizing the Sinclair S-1 miniature swine will allow it to be the model of choice for preclinical toxicology studies.
**1596 Cardiac Ultrastructure Damage and Pathological Remodeling after Halogen Inhalation in Rats**

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We have demonstrated that inhaled halogen gases cause extensive acute cardiac injury and dysfunction. Whether the acute injury persists and causes long-term effects in the heart is unknown. This gap in knowledge limits the potential for development of effective therapies. We sought to determine the role of halogen/bromine (Br) in causing delayed cardiopulmonary dysfunction and to establish the potential underlying mechanisms and effects on cardiac function and ultrastructure. Br, inhalation (600 ppm, 45 min) in adult Sprague Dawley rats caused acute (3-24h) increases in bronchoalveolar lavage fluid (BALF) protein and circulating troponin I, fatty acid binding protein (fHABP) and NT-proBNP. BALF protein returned to normal at 48h after exposure. However, troponin, fHABP and NT-proBNP remained elevated at 28d post exposure. Heart weight/body weight ratio was increased at all time points suggesting cardiac hypertrophy. Immunohistochemistry revealed extensive cardiac tissue damage and increased endocardial fibrosis. Significant increase in collagen was demonstrated by hydroxyproline assay in the bromine exposed rat hearts at 28 d after exposure. Echocardiography revealed significant diastolic and systolic dysfunction in rats surviving bronchoalveolar inination at 7-28 d after bromine inhalation. Thus, a single exposure of bromine may have persistent chronic adverse effects on the heart.

**1597 Maternal and Embryo-Fetal Historical Background Control Data in the Hsd: Sprague Dawley Rat**

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Maternal and embryo-fetal historical background control data supports improved interpretation of lesions in reproductive toxicity studies. A maternal and embryo-fetal toxicity study was performed to generate historical background control data in the Hsd:Sprague Dawley rat model. One hundred previously nulliparous and virgin time-mated female Sprague Dawley rats, allotted in four subsets of 25 rats each, were housed under standard housing and husbandry conditions with ad libitum access to a standard diet (18% protein, 6% fat) and water. Rats were administered tap water (10 mL/kg body weight) by oral gavage once daily from gestational day 6 to 17. Body weight, food consumption, and clinical observations were monitored throughout the in-life phase of the study. On gestational day 20, animals were euthanized and submitted for Cesarean section and necropsy. A macroscopic postmortem evaluation was performed on all animals, including counts of corpora lutea and implantations and uterine weights. Fetuses were removed, weighed, sexed, and examined externally for defects as well as soft tissue abnormalities and skeletal anomalies. Placentas were examined and weighed. One hundred dams were pregnant and 100 litters were evaluated. There were 1,488 fetuses examined externally; 698 had fixed visceral evaluations and 790 fetuses had skeletal evaluations. Detailed analysis of maternal data, including body weight, food consumption, and pregnancy outcome will be presented. Fetal data including uterine and placental weights, in addition to fetal examinations, including external, soft tissue (visceral), and skeletal will also be presented. These data support the use of the Hsd:Sprague Dawley rat as a valuable toxicity model and will assist in interpretation of lesion data in future studies with this model.

**1598 Strain-Chemical Curation at the Rat Genome Database**

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Rats have been used as experimental models for many decades to study physiological and pathological processes. To facilitate the use of rat data derived from these models, the Rat Genome Database (RGD) has endeavored in qualitative and quantitative curation of rat strain data using controlled vocabularies. The strain-associated antigens are integrated with genes and alleles for comprehensive query at the RGD. For example, from the hypertension disease model SS-Pleko7mHSd1/1, users can find the disease-associated gene Plekha7 mutant allele and associated phenotypes in qualitative terms or quantitative measurements. To expand the scope of strain curation, RGD is now launching a project to target rat strains and chemical/drug curation. The project is focused on curating mutant rats, either spontaneous or genome-edited, which exhibit drug/chemical-induced phenotypes different from wild type rats. Examples are the spontaneous LE/OIrBarth rat strain and the zinc-finger nucleases-induced F344-Nle21emm/++ rats. Their altered susceptibility to inducing agents is captured by associating inducing agents, rat strains, inductions, phenotypes and quantitative measurements for these phenotypes in the database. The incorporation of chemical-induced qualitative phenotypes and quantitative measurements of mutant rat strains at RGD will empower researchers to make the best use of publicly available data.

**1599 Evaluation of the Potential for Fibrous Talc to Cause Mesothelioma Based on Available In Vitro and In Vivo Animal Studies**

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Elongate mineral particles (EMPs), also sometimes called mineral fibers, refer to any mineral particle with a length-to-width ratio of at least 3:1 and a length ≥5 μm. Asbestos is an EMP and, depending on the type, has long been causally related to the development of mesothelioma in humans. Fiber dimensions, durability, and surface characteristics are thought to be some of the key determinants of asbestos carcinogenicity. Experiences with asbestos have generated interest in the potential for non-asbestos EMPs to pose cancer risk, including mesothelioma, in humans. Asbestos-like EMPs used in cosmetic applications have a predominantly plate-like (non-EMP) structure, while EMPs mined for industrial applications can be found in the form of long, thin fibers that meet the definition of an EMP and, further, can be of the more specific dimensions thought to be important for asbestos to cause mesothelioma. We have identified studies in which historical samples containing fibrous talc have been characterized for mineral composition and dimensions using various analytical methods (e.g., energy dispersive spectroscopy) and that have been employed alongside asbestos-containing samples in in vivo implantation and/or in vitro experiments designed to examine outcomes relevant to mesotheliogenic potential. These studies provide evidence that fibrous talc, including fibrous talc in samples where the dimensions are consistent with the most potent forms of asbestos for inducing mesothelioma, does not cause mesothelioma or other relevant outcomes in experiments where asbestosform amphiboles did. These findings are consistent with animal and epidemiology evidence in supporting that talc, unlike some types of asbestos, does not possess the important characteristics sufficient to cause mesothelioma. These findings also support that there is more to EMP pathogenicity than fiber dimensions, such as mineral type and crystal structure.

**1600 Intratubine Infusion of AMD3100 in Ewes: Elucidating the Importance of CXCL12 at the Fetal-Maternal Interface**

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Early pregnancy is characterized by complex interactions between fetal trophoblast cells and maternal endometrium, which direct major peri-implantation events including localized inflammation and endometrial modifications to establish proper placental development. Because pro-inflammatory mediators are important for conceptus interdigitation in endometrium, a better understanding of the molecular pathways regulating this localized inflammation is needed to advance knowledge of the process by which the endometrium becomes receptive to embryonic implantation. We identified CXCL12 as a critical driver of placental angiogenesis, but this pleiotropic chemokine and its receptor, CXCR4, are also important to cell survival and proliferation as well as inflammation, and may facilitate uterine receptivity. As such, we hypothesized that CXCL12 regulates endometrial inflammation during implantation in ewes. We demonstrated that CXCL12 stimulates TNF production in cultured ovine endometrial cells. Further, to establish functional implications of CXCL12 at the fetal-maternal interface, we developed an in vivo model to inhibit local signaling of this chemokine-receptor pair using a potent CXCR4-specific inhibitor, AMD3100. Osmotic pumps were surgically installed on day 12 of gestation in sheep and delivered AMD3100 or PBS directly into the uterine lumen ipsilateral to the corpus luteum for 7 days. On day 20 of pregnancy, endometrial tissues were collected and evaluated for inflammatory mediators and endometrial modifications using western blotting and immunohistochemistry. Post-infusion, protein abundance of TGF81 in aglandular endometrium was less (p<0.05) in ewes receiving AMD3100 than control, while synthesis of all other cytokines assessed was similar. Conversely, glandular endometrial expression of IL12 and IFNG following local AMD3100 infusion. Immunohistochemical observation of endometrial epithelial morphology revealed a persistence of co-
1601 A Six-Month Subcutaneous Infusion Qualification in Beagle Dogs

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The ability to continuously administer drugs subcutaneously is critical to attain appropriate exposure of some pharmaceuticals which offer advantages over traditional administration routes in preclinical development models. The objective of this study was to assess the tolerance and background observations following continuous subcutaneous infusion of saline via a surgically implanted catheter in Beagle dogs for up to 6 months. Five male and five female Beagle dogs underwent surgical implantation of a medical grade subcutaneous catheter. The tip of the catheter was positioned in the lumbar region and the catheter was exteriorized at the dorso-cervical region through a subcutaneous tunnel via a cath-in-cath/port system. Normal saline was continuously infused at a rate of 0.1 mL/hour generally for 1 month (3/sex) or between 4 and 6 months (2/sex). Animals were evaluated for clinical signs, body weight, limited clinical pathology and histopathology. The dorso-lumbar area facilitated clinical evaluation of the dosing site and the infusion rate was achieved with dosing accountability values generally within acceptance criteria (+/- 5%). The animals showed normal bodyweight gain, and limited clinical pathology changes. Results obtained from this study provided sufficient evidence that the surgically implanted catheter was well tolerated and did not result in unexpected background changes other than those normally observed with surgically implanted catheters. Specifically, a mixed cellular inflammatory reaction was observed surrounding the subcutaneous catheter infusion site. There was no difference in the inflammatory reaction following saline infusion over a period of 4, 5 or 6 months.

1602 Evaluation of Aflatoxin B1-Induced Human Hepatotoxicity in Humanized Liver Chimeric Mice and Fresh Human Hepatocytes Isolated from Chimeric Mice

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Chimeric mice with humanized livers have been considered a useful in vivo model for not only drug metabolism and pharmacokinetics studies, but also for the prediction of human hepatotoxicity. Recently, we reported that fresh primary human hepatocytes isolated from chimeric mice (PX-B cells) showed high platability and could be cultured for several weeks with high hepatic function in a conventional 2D culture condition. In the present study, we examined whether humanized liver mice and PX-B cells have the potential to be used for evaluation of human hepatotoxicity induced by aflatoxin B1 (AFB1), a well-known compound that shows species-specific hepatotoxicity. First, control (CD1) mice and CDNA-uPA/SCID mice with humanized livers showing high replacement index (>70%) were administered corn oil, AFB1 (3 mg/kg), and carbon tetrachloride (CCL4, 50 mg/kg) respectively for 7 days. CCL4 treatment induced increase in serum ALT activity and body weight loss in both control mice and humanized liver mice. In contrast, AFB1 treatment promoted the elevation of serum ALT activity and body weight loss in chimeric mice with humanized livers, but not in control mice. The results of human ALT1-specific ELISA revealed that significant increase in serum human ALT1 was induced by AFB1 treatment but not CCL4. Consistent with these results, histological examination indicated that AFB1 treatment promoted vacuolization in human hepatocytes, but not in mouse hepatocytes in the chimeric mice livers. To examine AFB1 toxicity, PX-B cells were cultured with or without AFB1 for 7 days. The cytotoxicity of AFB1 was evaluated by real-time cell analysis (RTCA) and WST-1 assay. The results of RTCA indicated that AFB1 showed dose-dependent cytotoxicity to PX-B cells, and the LC50 value on day 3 (0.83 μM) was comparable to that of primary human hepatocytes reported in previous studies. The results of WST-1 assay suggested that 7 days of AFB1 treatment showed more severe cytotoxicity to PX-B cells than treatment with AFB1 for 1 or 3 days. Treatment with aminobenzotriazole (500 μM), a nonspecific CYP inhibitor, could reduce AFB1 cytotoxicity, indicating that AFB1 metabolite(s) reduced the viability of PX-B cells. These results suggest that chimeric mice with humanized livers and PX-B cells are useful in vivo and in vitro tools to predict human hepatotoxicity, respectively.

1604 Electronic Waste: An Evolving Global Health Concern and Risk Assessment Challenge

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As the demand for electronics increases, the amount of electronic waste (e-waste) steadily accumulates at a rapid pace. An estimated 65 million tons of e-waste were created globally in 2017, with further increases projected in the years ahead. E-waste is composed of an alarming combination of several hazardous substances. A systematic review looking at health outcomes related to e-waste exposure showed that increases in spontaneous abortions, stillbirths, and premature births, and reduced birth weights and birth lengths, are associated with exposure to e-waste. Direct and indirect exposures are a threat to human health and vulnerable groups such as fetuses, children, pregnant women, the disabled, and workers in the informal sector. Because of this threat, they need specific protection. The majority of e-waste recycling is conducted in low-to-middle-income countries informally, by workers using primitive techniques such as burning, with little or no safeguards in place for human and environmental health. This session will provide an overview of the e-waste problem and present research findings from studies conducted in India and Vietnam. The session will end with a presentation that will discuss the multi-factorial problem of e-waste due to limited studies and the multiple routes of exposure to multiple classes of hazardous substances in the context of vulnerable populations. These presentations will inform a panel that will discuss risk assessment challenges (exposures to a mixture of chemicals from multiple sources) and provide a forum to discuss strategies to reduce exposures to e-waste.
1605 Federal Efforts in Rapidly Assessing Hazard and Risk to Emerging Threats and Emergency Response

M. J. Devito, NIEHS/NTP, Research Triangle Park, NC.

Under the authorization of a variety of federal statutes, several federal agencies are involved in protecting public health from emerging environmental threats and in emergency response. These federal agencies have developed programs that can provide regulators with a rapid assessment of the potential hazard and risk associated with exposures in times of emergency response. These approaches combine rapid literature assessments, computational toxicology, and in vitro toxicology. These same approaches are also being applied to evaluate emerging issues related to chemical exposures. This session will highlight these efforts at the US Department of Defense, the Agency for Toxic Substances and Disease Registry, the US EPA, and the NIEHS National Toxicology Program.

1606 Immune-Epithelial Cell Crosstalk in Lung Toxicology and Disease

A. Venosa, University of Pennsylvania, Philadelphia, PA.

Understanding the mechanisms involved in mediating the pollutant-based deficits in pulmonary health remains an area of continued interest within SOT and in the field of public health. The lung represents a unique toxicological target due to its continuous exposure to the gaseous components of the environment and its function as a major first pass organ. The environment of the lung surface is a unique system consisting of both barrier and immunological defenses. A key aspect of the system is the interaction between resident cells of the lung (both parenchymal epithelial cells and resident immune subsets) and recruited inflammatory cells. It is the goal of this session to address the molecular mechanisms involved in the resident/recruited response to toxicants. Therefore, the session will highlight current developments in the fields of lung biology, immunotoxicology, and pharmacology. The pulmonary response to toxicant exposure consists of a number of complex processes, including direct and indirect inflammatory, injury, inflammation, and resolution. Much of this response is regulated by the resident cells of the lung surface, including both type I and type II epithelial cells and the alveolar macrophages. Proper recruitment and activation of circulating inflammatory cells is essential to mount the appropriate inflammatory and resolution responses. Therefore, epithelial-immune crosstalk is critical in the regulation of the pulmonary response to toxicants. To this end, high-throughput phenotyping of resident and infiltrating cells has highlighted novel pathways involved in cell-cell communication during progression and resolution of lung injury.

1607 Alveolar Type II Cells Initiate and Modulate Inflammation in Lung Surfactant

A. Venosa, University of Pennsylvania, Philadelphia, PA.

The missense isoleucine to threonine substitution at position 73 (I73T) in the Surfactant Protein-C (SP-C) gene, an alveolar type-2 cell (AT2) restricted protein, has been linked to sporadic and familial cases of idiopathic pulmonary fibrosis (IPF). This disease is associated with intermittent inflammatory episodes, termed “acute exacerbations”, marked by altered gas exchange, alveolitis, and diffuse alveolar damage. To model lung injury resulting from epithelial AT2 cells dysfunction, we generated an inducible SP-C-I73T transgenic mouse. Tamofoxifen administration to SP-C-I73T mice resulted in early increases in mutant spfctc73T mRNA and proSP-C-I73T protein expression accompanied by diffuse lung injury, vascular leak, increased BAL cell counts, and significant mortality. By 6wk, lungs of surviving SP-C-I73T mice showed marked alveolar septal thickening, diffuse collagen deposition, and foci of aSMA+ cells consistent with IPF phenotypes. The immunohistochemistry analysis of AT2 from SP-C-I73T mice revealed early and persistent (3-14d) epithelial expression of a wide array of recruitment factors including MCP-1, IL-5, Eotaxin, IL-6, CCL-17, and KC, findings corroborated by ELISA analysis of lung BAL. Flow cytometry was adopted to temporally characterize the effects of AT2 cell dependent recruitment on effector cell crosstalk. SP-C-I73T induction was associated with significant reduction in SiglecFhiCD11bloC64hiCD11chi resident macrophages, paired with transient (3-7d) reduction in expression of the antiinflammatory receptor CX3CR1. This was mirrored by sequential accumulation of Ly6C+ monocytes (3d), Ly6G+ neutrophils (7d) and SiglecFhiCD11blo eosinophils (2wk) in the lung. PCR analysis of BAL cells and tissue immunohistochemistry confirmed phenotypic shift from proinflammatory (iNOS and IL-6) at 3d, to an antiinflammatory/profibrotic activation state (Arg1, FIZZ-1) at 2wk. Pharmacological depletion of resident macrophages (ltd.) and infiltrating monocytes (i.o.) with clodronate liposomes revealed that resident immune cells are protective in epithelial cell injury, while peripheral Ly6Chi monocyte may worsen injury outcome. These data demonstrate that this IPF model is a valid tool for examining epithelial/inflammatory crosstalk and thus provides a novel tool to characterize the inflammatory response to toxicant induced epithelial cell injury.

1608 Extracellular Vesicle: An Emerging Mediator of Intracellular Crosstalk in Lung Inflammation and Injury

Y. Jin. Boston University School of Medicine, Boston, MA. Sponsor: A. Venosa

Acute lung injury (ALI) / acute respiratory distress syndrome (ARDS) is a highly complex process which can be triggered by various stimuli, including both non-infectious (sterile) and infectious ones. Inflammatory lung responses are one of the key features in the pathogenesis of this devastating syndrome. How ALI/ARDS associated inflammation is developed remains incompletely understood, particularly after exposure to sterile stimuli. Emerging evidence show that extracellular vesicles (EVs) regulate intercellular communications and inflammatory responses in various diseases. We characterized the generation and function of pulmonary EVs in the setting of ALI/ARDS, induced by sterile stimuli (oxidative stress, cigarette smoke or acid aspiration) and infections (LPS/gram negative bacteria). EVs detected in the broncho-alveolar lavage fluids (BALF) were highly upregulated after exposure to both types of stimuli. After sterile stimuli, alveolar type-1 epithelial (ATI) cells were the main source of the BALF EVs. Interestingly, infectious stimuli-induced BALF EVs were mainly derived from alveolar macrophages (AMs), rather than epithelial cells. Functionally, BALF EVs generated in both the non-infectious and infectious ALI models promoted the recruitment of macrophages. Furthermore, BALF EVs differently regulated the cytokine productions, TLR expressions and inflammatory mediators in AMs in vivo. Regardless of their sources of generation, BALF EVs significantly contributed to the development of lung inflammation in both the “sterile” and “infectious” ALI. Collectively, our results provide novel insights on the mechanisms by which EVs regulate the development of lung inflammation in response to sterile “infectious” stimuli, potentially providing novel therapeutic and diagnostic targets for ALI/ARDS.

1609 To Each Their Own: Molecular Mechanisms of Inter-Individual Variability in the Effects of Inhaled Toxicant Exposure

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Traditional approaches to identifying susceptible populations have relied on factors such as age, genotype, and disease status to explain variability in exposure outcomes; however, these are neither sufficient to faithfully identify
differentially responsive individuals nor are they modifiable factors that can be leveraged to mitigate the effects of toxic exposures. The epigenome is dynamic and shaped by an individual’s environment. We previously determined that the ozone-mediated induction of pro-inflammatory genes IL-8, IL-6, IL-1a, and IL-1β is dependent on the concerted activation of the EGRF/MEK/ERK and MKK4/p38 mitogen activated protein (MAP) kinase pathways in primary human bronchial epithelial cells (pBECs). We then validated these findings in vivo by comparing the activation of cellular networks in bronchial epithelial cells collected from healthy volunteers following controlled exposure to filtered air or ozone. As effector kinases, activated ERK and p38 translocate into the nucleus to drive changes in gene expression. We hypothesized that the impact of these signals on exposure-induced gene expression was mediated by epigenetic modification states within the regulatory regions of exposure-induced genes. We then characterized the relationship between the baseline abundance of six epigenetic markers with established roles as key regulators of gene expression - trimethyl histone H3 lysine 4, acetyl H3K27, p38/MEK/ERK phosphorylated (p38/MEK/ERK), acetyl/ubiquitylated histone H3 (H3K27, polyubiquitinylated H3), dihydroxy methyltetrahydrofolic acid (dihydroxy methyltetrahydrofolic acid) - and the variability in the O3-induced expression of inflammatory and oxidative stress genes in an ALI model using pBECs from a panel of donors. The relationships that we observed led to our proposal of the “Epigenetic Seed and Soil” model in which the baseline abundance of particular chromatin modifications within the regulatory regions of specific toxicant-responsive genes correlates with the magnitude of their exposure-mediated induction. The model is also applicable to the use of baseline epigenetic data to predict exposure responses in cell and tissue types throughout the body. Identifying the role of the epigenome in toxicant responsiveness will provide an additional dimension to our understanding of the mechanisms underlying inter-individual variability in exposure effects and provide new insights into identifying populations.

1612 Patterns of Co-exposure and Its Implications for Understanding the Health Effects of Mixtures

T. Webster. Boston University School of Public Health, Boston, MA.

While current chemical testing tends to focus on individual chemicals, the exposures that people actually experience involve mixtures of chemicals. The number of mixtures that can be formed from the thousands of environmental chemicals is enormous, and testing all of them would not be realistic. In recent years, the ongoing revolution in exposure science and analytic chemistry (e.g., non-targeted analysis) is permitting better assessment of exposures to more and more chemicals at lower cost. It appears likely that we will be facing the biggest data challenge in exposure science in the not very distant future, and novel statistical methods are needed for analyzing these data. Collaboration between mixtures toxicologists and exposure scientists has great promise. Exposure science has a very important role to play by (1) determining the combinations of chemicals to which people are actually exposed, removing the limiting problem facing toxicologists; (2) identifying highly correlated exposures that might be better studied using whole mixtures methods than component-based methods; and (3) providing information needed by epidemiologists studying exposure to mixtures. A critical problem is understanding the patterns of exposure; for example, which exposures tend to occur together and how does this tendency depend on demographic and other factors? This session will bring together exposure scientists and mixtures toxicologists to examine methods for analyzing patterns of co-exposures; apply them to large datasets, such as National Health and Nutrition Examination Survey (NHANES) biomonitoring data and personal care product purchasing database; and discuss their implications for research on the health effects of exposure to mixtures in toxicology and epidemiology.

1610 Mechanisms of Exposure-Based Injury in the Resident Tissues of the Lung

L. Van Winkle. University of California Davis, Davis, CA.

Lung injury and repair incorporates complex interactions that integrate across the multitude of tissues and cells in the lung. Key to these responses are integrated signals between immune cells and the lung epithelium. In the lung there are two anatomically distinct epithelia: conducting airways and alveolar epithelia. The conducting airway epithelia has a unique composition and consists of a subpopulation of sensory cells such as Club and Clara cells that sit on a layer of extracellular matrix surrounded by attenuated fibroblasts forming a unit called the epithelial mesenchymal trophic unit. Similarly the alveolar compartment consist of AT1 and AT2 epithelial cells that are in close apposition to fibroblasts but also endothelium. Frank epithelial toxicity is a component of the in vitro exposure-based injury and can be influenced by cell type, cell state (age, sex, prior exposure, antioxidant status) and location within the lung. In mice exposed acutely to inhaled naphthalene, age and sex influence the degree of injury, with female exhibiting 4 fold more epithelial damage in proximal airways at 3 weeks of age than at 1 week of age, while males are protected. The age at time of exposure can also significantly influence the degree of injury following inhalation of particulate matter and this has been shown to be related to the ability of the epithelium to generate an effective anti-oxidant response. For example, neonatal rats are more susceptible to toxicity from ultrafine PM leading to decreases in airway epithelial glutathione pools that are not seen in adult rats. In addition to outright toxicity, exposure can change the steady state of the lung and initiate metaplasia, proliferation and regeneration of tissue components. Mucous metaplasia in rodents is a common response to ozone exposure. For epithelial cells in the lung one of the earliest changes in response to injury is in the distribution, abundance and type of secretory protein production which is regulated not only by the toxicant but also by the type and state of inflammation. Regulation of secretory protein expression is well understood in surfactant protein D expression. Specific examples from injury and repair models involving a variety of toxic exposures including nanoparticles, ozone, naphthalene and PM will be used to support key points.

1611 Inflammatory Responses of Resident and Recruited Immune Cells to Inhaled Toxicants

K. Gowdy. East Carolina University, Greensboro, NC.

Macrophages and monocytes are present in tissue as distinct populations with unique functional roles. In the lung, the macrophage populations include alveolar and interstitial macrophages whereas during inflammation, there is an influx of exudative/infiltrating macrophages that are derived from circulating monocytes. During tissue injury and inflammation, these myeloid cells have diverse and nonredundant roles in both the initiation of inflammation as well as the resolution through their production of lipid mediators. Evolving research has provided insight that inhaled toxicants (ozone, cigarette smoke, particulate matter) can alter the pulmonary lipid metabolism, producing more proinflammatory lipid mediators (prostaglandins, leukotrienes) and suppressing the production of specialized proresolving lipid mediators (protectins, maresins, resolvins). Exposure to inhaled toxicants, can specifically increase the alveolar macrophage release of proinflammatory lipid mediators, however the lipid metabolism of other macrophage/monocyte subpopulations after toxicant exposure is currently understudied. Therefore, identifying the consequences of inhaled toxicants on the lipid metabolism of specific pulmonary macrophage and/or monocyte subsets will define mechanisms behind how these toxicants can modulate pulmonary immunity and inflammation. This session will highlight the role of lipid metabolism for pulmonary macrophage/monocyte subsets and their lipid metabolism in lung injury and repair in multiple models of inhaled toxicant exposure. Currently, an avenue of research that we are pursuing is investigating pulmonary lipid metabolism after ozone exposure, with emphasis on the production of specialized pro-resolving lipid mediators. Our data indicate that ozone exposure suppresses the pulmonary production of specialized proresolving lipid mediators, that is most pronounced in the interstitial macrophage population. These data as well as those by other laboratories link immune cell function with lipid metabolism to provide mechanistic insights for potential therapeutic interventions.
1614 Hierarchical Structure of Patterns of Correlations between Biomarkers of Exposure and Their Implications for Studying the Health Effects of Mixtures

T. Webster, Boston University School of Public Health, Boston, MA.

This presentation will provide a short introduction to the symposium and then discuss the use of hierarchical clustering to examine patterns of correlations amongst exposures. We computed correlation coefficients between biomarkers in NHANES (the ongoing National Health and Nutrition Examination Survey) and several other general population cohorts. We defined a distance metric based on correlation coefficients and applied agglomerative clustering using average linkage. The resulting data were displayed using a heat map of the clustered correlation matrix. Results were striking: several cohorts show a block diagonal structure indicating that some groups of compounds tend to be highly correlated within groups but have low correlation with each other. Potential reasons for this pattern include commonalities (and differences) of physical chemical properties and exposure sources. These results suggest that health studies of such groups of compounds should not confound each other but may still have the possibility for interaction.

1615 Market Basket Data as a Tool to Sharpen Aggregate and Cumulative Chemical Risk Assessment


Surveys to acquire information on consumer product use, including use of household cleaning products and personal care products (PCP), can provide valuable information on potential chemical exposures and population habits and practices associated with these products. However, surveys take considerable effort to conduct and may become dated over time as new products emerge and markets change. Market basket data offers a potentially efficient mechanism to capture current product use patterns. Frequent itemset mining and association rule mining were used to analyze a database of PCP purchases for sixty thousand households over a one-year period in 2012. Robust co-occurrence patterns and associations were found for several PCP product categories and were consistent with use surveys. Furthermore, PCP purchase patterns varied by demographics explored (race, education, income, and family composition). We conclude that purchase data can fill a critical data gap when use survey data is absent, and can inform aggregate and cumulative risk assessment when coupled with product ingredient databases.

1616 Identification of Prevalent Exposure Biomarkers in the US Population Using NHANES

D. Kapraun, and J. Wambaugh, US EPA, Research Triangle Park, NC.

This presentation will examine a second approach to analyzing NHANES data using frequent itemset mining (FIM), a technique traditionally used for market basket analysis. We analyzed data from the 2009–2010 cycle of the continuous National Health and Nutrition Examination Survey (NHANES) to identify combinations of chemicals that frequently co-occur in people. We identified 90 chemical combinations consisting of relatively few chemicals that occur in at least 30% of the U.S. population. Certain combinations were far more prevalent in vulnerable populations, for example the combination of monoisobutyl phthalate and mono-n-butyl phthalate was much more prevalent in children than in the general population. This work demonstrated how FIM can be used in conjunction with biomonitoring data to narrow the large number of combinations of chemicals that could be formed from NHANES chemicals down to a smaller number of prevalent chemical combinations.

1617 Understanding and Predicting Patterns of Co-exposure

R. Zaleski, ExxonMobil Biomedical Sciences, Inc., Annandale, NJ. Sponsor: T. Webster

Biomonitoring data has proven very useful for understanding what substances an individual may be co-exposed to, and it can also be used to determine the relative magnitude for each of the constituents comprising the co-exposures. For example, NHANES co-exposure patterns for an analysis of several phthalate esters demonstrated that at upper percentiles of total exposure typically one ester dominates an individual’s exposure, but at median total exposure levels multiple esters contributed. Co-exposure insights that can be obtained from biomonitoring data to date have been limited to the domain of the biomonitoring data set; i.e., observations can be made only for those chemicals for which biomonitoring data are available. The ability to extend our understanding to co-exposure patterns for substances beyond current biomonitoring sets will be an important predictive capability. Could a combination of physical chemical properties, toxicokinetics, uses (exposure source) and function lead to this? Expanding current biomonitoring studies to include additional contextual information will be important to develop and achieve this capability. Further, given the wide range of co-exposure combinations that can occur from natural and man-made sources, the ability to focus in on co-exposures that are most meaningful for health assessment is needed.

1618 Using Pregnancy Cohort Data to Identify Human-Relevant Mixtures for Experimental Evaluation in a Whole Mixture Risk Assessment Strategy

C. Gennings, Icahn School of Medicine at Mount Sinai, New York, NY.

Experimental studies of mixtures are generally limited to convenient single chemical classes, while real human exposure is clearly not thus limited. In this presentation we will take a whole mixture strategy using the following four steps in a case study of mixtures of endocrine disrupting chemicals (EDCs): (1) identify single chemicals in human data of EDCs - measured in blood/urine of pregnant women in a cohort study that are associated with neuro-developmental effects in children; (2) define and construct a “typical” mixture from the cohort consisting of the “bad actors” identified in step 1; (3) experimentally test this reference mixture in an in vivo model to estimate a dose response relationship and determine a point of departure (POD) associated with an adverse health outcome; and (4) for the subpopulation of pregnant women determined to have a sufficiently similar mixture to the reference mixture, generate a “similar mixture risk indicator” (SMRI) to determine the level of concern for exposure relative to the POD. The SMRI is finally used to demonstrate the relative risk of adverse neurodevelopment as measured by this metric of risk assessment.

1618a An Evaluation of HTS and Mixtures: From Component-Based to Whole Mixtures Studies

M. DeVito, NIEHS/NTP, Research Triangle Park, NC.

One of the challenges in understanding mixtures is the huge number of possibilities. Clearly, traditional testing methods alone cannot provide a means to address the mixtures issues. In Tox21 we have studied both component based approaches as well as whole mixture approaches using high throughput screening (HTS) approaches. While the HTS approach has the advantage in throughput, it also has limitations. The challenge to HTS approaches is that “do-overs” are exceptions. Once 10,000 substances and mixtures are screened, it is rare to go back and re-run a couple dozen that did not have adequate dose response information. Thus, dose selection becomes critical in the study design to ensure that the dose range selected provides adequate dose response information. In addition, pairing mixtures HTS results with sufficient HTS chemical analysis of the stability of the mixtures is critical in ensuring that the HTS results can be adequately interpreted. Despite these limitations, a major advantage of the HTS approach is that it allows for preparing numerous mixtures that include mixing ratios of the chemicals that span possible human exposures as well as mixtures that test the boundaries of our dose addition models. Testing both relevant human exposures and the boundaries of our models will lead to more accurate hazard and dose response assessments of mixtures.
1619 Scaling Barriers: Cellular Dynamics and Models of Blood-Brain Barrier Developmental Toxicity

K. Saili, US EPA, Research Triangle Park, NC.

This session will focus on a critical vascular interface, the blood-brain barrier (BBB), with regard to embryology and toxicology. The BBB is a core of the neurovascular unit (NVU) comprising microvascular endothelial cells, pericytes, astrocytes, microglia, and neurons. These cell types function in various capacities throughout development to regulate the distribution of substances from the circulatory system to the developing brain (i.e., toxicokinetics). Although historically described as “leaky,” the leading perspective in the field has recently shifted toward an understanding that the BBB is functional and intact as it forms. BBB research tends to focus on toxicokinetics, but less is known about the toxicodynamic impact that drugs and chemicals may have on the developing BBB. Moreover, it is unclear whether such impacts would lead to developmental neurotoxicity (DNT). Evidence from mouse models and human genetics suggests that a model of BBB development and function has a role in the etiology of neurobehavioral disorders such as autism spectrum disorder, supporting the hypothesis that chemical disruption of the developing BBB may also lead to DNT. While current models representing the state-of-the-science in this field have not demonstrated a direct link between BBB perturbation by chemical exposures and subsequent DNT, this hypothesis remains to be adequately tested. Alternative models may provide tools toward understanding this “black box” in BBB toxicity. This session will address the integrative biology and systems toxicology underlying BBB toxicodynamics and highlight the cutting-edge in vivo, in vitro, and in silico models currently utilized for early life-stage considerations. The presenter lineup will begin with an overview that frames the importance, yet paucity, of developmental BBB research, followed by talks progressing from in vivo to in vitro and in silico BBB models. The first Co-Chair will provide an introduction to the session theme by describing key cell types and timing of embryonic BBB development across species. This introduction will cover other areas of the brain (e.g., circumventricular organs) and their barriers; provide an overview of the state of the art in BBB models that will be described in more detail by the session presenters; and briefly survey traditional, toxicokinetic models and BBB transporters. The first presentation will introduce the cortical BBB and describe a mammalian in vitro model to investigate the role of microglia in establishing BBB integrity during embryonic development. The next presenter will discuss an embryonic, transgenic zebrafish model being used to investigate the role of pericytes in mediating developmental BBB toxicity. The next presenter will introduce a 3D in vitro model designed to test BBB permeability to therapeutic antibodies. The final presentation will discuss novel multiscale in silico models for unscrambling complex cellular dynamics of BBB development in a computational neurovascular unit (cNVU) system, whereby toxicity pathways interact with fundamental morphoregulatory signaling (e.g., Wnt, Shh, Delta/Notch) during windows of vulnerability to developmental neurotoxicants. To wrap up the session, the second Co-Chair will emphasize the importance of establishing alternative BBB models that reduce animal testing, in addition to providing translational context for developmental BBB research by discussing the importance of these studies in relation to children’s environmental health protection.

1621 Environmental Contaminant Exposure Reduces Pericyte Coverage of the Developing Cerebral Vasculature

J. Plavicki, and M. Yue. Brown University, Providence, RI; and University of Wisconsin - Madison, Madison, WI.

Pericytes are a critical component of the blood brain barrier (BBB) that help maintain vascular stability and also modify barrier properties of brain endothelial cells by influencing efflux transporter expression. Correspondingly, loss of pericyte coverage is associated with cerebral hemorrhage and disrupted BBB development. Exposure to tetrachlorodibenzo-p-dioxin (TCDD) is known to produce hemorrhaging in the brain, and the periphery. However, why TCDD exposure produces hemorrhaging is unknown. To examine whether loss of pericyte coverage could contribute to the observed hemorrhaging phenotype, we exposed double transgenic embryonic zebrafish carrying fluorescent reporters for pericytes and endothelial cells (tdgpf-Il:GFP, kdrFl:dsRed2) to TCDD and counted the number of pericytes present in the midbrain and hindbrain. We found that a 1 part per billion and a sub-lethal 50 parts per trillion exposure resulted in a significant reduction in pericytes in the developing brain. To determine whether pericyte-specific activation of the aryl hydrocarbon receptor (AhR) is sufficient to produce hemorrhaging, we are using a pericyte-specific transgenic line to drive the expression of a constitutively active and corresponding control. In addition, we are examining whether pericyte-specific AhR activation alters BBB permeability. This presentation will also explore the application of the embryonic zebrafish BBB model to identify additional putative BBB disrupting compounds and further investigate the mode of action by which they elicit developmental neurotoxicity.

1622 BBB On-a-Chip: 3D In Vitro Models of the Human Blood-Brain Barrier


The blood-brain barrier (BBB) ensures a homeostatic environment within the central nervous system and protects the brain from harmful substances. At the same time, it largely prevents therapeutics from crossing, thereby making it difficult to treat brain diseases. Moreover, the BBB plays a crucial role in initiating or aggravating damage to brain tissue, for instance in multiple sclerosis, stroke, or Alzheimer’s disease. Restoration of BBB function is therefore a promising therapeutic avenue. To study mechanisms of barrier function and to explore approaches for improved drug delivery, we established two microfluidic 3D cell culture models of the BBB, both of them comprising human brain microvascular endothelial cells, pericytes, and astrocytes. The first model, developed by Mimetas, uses a 3-lane OrganoPlate® based on a 384-well microtiter plate and allows for parallel culture of 40 perfused microvessels. The second model, utilizing Nortis technology, closely mimics the multicellular anatomy of the in vivo BBB in a 3D tubular microvessel perfused under physiological flow rates. We supplement our BBB toolkit by the simple but scalable Transwell system, traditionally used in the field. In all models, we incorporate cells of human origin, either immortalized lines or derived from induced pluripotent stem cells. In addition to validating the BBB phenotype by immunocytochemistry and transcriptomic profiling, we confirmed barrier function and adopted assays to demonstrate receptor-mediated transport of antibodies, dynamics of leucocyte-endothelial interactions, and pharmacological modulation of barrier permeability. Moreover, these systems could be adapted to screen chemical libraries to identify toxicants that may impact BBB integrity by promoting a pro-inflammatory environment. These novel human, thus translatable BBB models provide a valuable toolkit for both high-throughput screening and detailed characterization of BBB function, supporting fundamental BBB research, drug discovery, and study of neurological disorders.

1620 Applying a Mouse Model of Embryonic Macrophage Depletion to Elucidate the Role of Microglia in Blood-Brain Barrier Development

K. Saili, US EPA/NCCT, Research Triangle Park, NC.

Microglia, the resident brain macrophage population, communicate with other cell types of the neurovascular unit (NVU) during normal brain development. We used a mouse model of embryonic macrophage depletions to test the hypothesis that microglia are essential for proper blood-brain barrier (BBB) formation. Macrophage depletion was achieved by injecting anti-colony stimulating factor 1 receptor (a-CSF-1R) antibody at 6.5 and 7.5, when yolk-sac macrophages are being generated, thereby transiently blocking the CSF-1 signaling pathway required for maintenance and differentiation of yolk-sac macrophages (microglial precursors). Flow-cytometry and immunohistochemistry indicated that this procedure specifically affects yolk-sac macrophages, thereby leading to a dramatic depletion of microglia as well as other embryonic macrophages throughout embryogenesis. To test whether macrophage depletion impacts BBB permeability, 70 kDa FITC-Dextran was injected into the hearts of embryonic macrophage-depleted fetuses and fluorescence was measured in the brain. Fluorescence imaging indicated a greater amount of FITC-Dextran diffusion in macrophage-depleted brains compared to wildtype controls, suggesting reduced BBB integrity and confirming a role in BBB development and function. This new mouse model will facilitate a greater understanding of the role of microglia in orchestrating BBB formation and function during embryonic brain angiogenesis, and will be used to test whether putative neurovascular toxicants impact NVU formation through a microglia-dependent process.
Development of a functional blood-brain barrier (BBB) is a complex process regulated by multiple cell types at different developmental stages. One important aspect of the developing neurovascular unit (NVU) involves interaction between invading endothelial cells, neuroprogenitor cells comprising the subventricular plexus, and microglia, the resident macrophages of the central nervous system (CNS). Microglia serve as an important mediator between neurotoxicology and immune function and dysregulation of these cells during development is hypothesized to disrupt BBB formation via decreased vessel branching and compromised vessel stabilization. To address the complexities of chemical exposure on the developing BBB, a cell agent-based model (ABM) was developed to predict the formation of the NVU with specific focus on the interaction between invading endothelial cells and microglia. Building upon a previous model of vasculogenesis, the current ABM characterizes the complex interaction between microglia and invading endothelial cells by incorporating multiple signaling pathways (Notch/dll4, CSF-1, VEGF-A, VEGF-C, sFlt1) in a biological framework. Molecular targets in the ABM were linked to signatures of neurovascular disruption through in vitro assay results in US EPA’s ToxCast high throughput screening dataset. Specifically, colony stimulating factor 1 receptor (CSF-1R) served as a marker of microglia abundance in the developing neuroepithelium. Concentration response effects using the NVS, ENZ, hCSF-1R assay in ToxCast were translated to chemical-specific effects on microglia abundance. Simulations of chemical-induced reduction in microglia resulted in a compromised NVU vasculature represented by decreased blood vessel branching and sprouting, which mimicked in vivo reports. The integration of in vitro data with in silico models provides a path forward to mechanistic prediction of developmental neurovascular toxicity and demonstrates a methodology for screening chemicals in silico and validating these biological paradigms using cell-based in vitro assays. Further development of the NVU will facilitate predictions of perturbations to the complex biology of the developing NVU from available in vitro data and predict disruptions in BBB viability. Declaration: This work does not reflect US EPA policy.

This session will focus on a critical vascular interface, the blood-brain barrier (BBB), with regard to embryoology and toxicology. The BBB is a core of the neurovascular unit (NVU) comprising microvascular endothelial cells, pericytes, astrocytes, microglia, and neurons. These cell types function in various capacities throughout development to regulate the distribution of substances from the circulatory system to the developing brain (i.e., toxicokinetics). Although historically described as “leaky,” the leading perspective in the field has recently shifted toward an understanding that the BBB is functional soon after it forms. BBB research tends to focus on toxicokinetics, but less is known about the toxicodynamic impact that drugs and chemicals may have on the developing BBB. Moreover, it is unclear whether such impacts would lead to developmental neurotoxicity (DNT). Evidence from mouse models and human genetics suggests that altered BBB development and function have a role in the etiology of neurobehavioral disorders such as autism spectrum disorder, supporting the hypothesis that chemical disruption of the developing BBB may also lead to DNT. While current models representing the state-of-the-science in this field have not demonstrated a direct link between BBB perturbation by chemical exposures and subsequent DNT, this hypothesis remains to be adequately tested. Alternative models may provide tools toward understanding this “black box” in neurotoxicology. This session will address the integrative biology and systems toxicology underlying BBB toxicodynamics and highlight the cutting-edge in vivo, in vitro, and in silico models currently utilized for early life-stage considerations. The presenter lineup will begin with an overview that frames the importance, yet paucity, of developmental BBB research, followed by talks progressing from in vivo to in vitro and in silico BBB models. The first Co-Chair will provide an introduction to the session theme by describing key cell types and timing of embryonic BBB development across species. This introduction will also briefly cover other areas of the brain (e.g., circumventricular organs) and their barriers; provide an overview of the state of the art in BBB modeling; and be described in more detail by the session presenters: and briefly survey traditional, toxicokinetic models and BBB transporters. The first presentation will introduce the cortical BBB and describe a mammalian in vivo model used to investigate the role of microglia in establishing BBB integrity during embryonic development. The next presenter will discuss an embryonic zebrafish model being used to investigate the role of pericytes in mediating developmental BBB toxicity. The next presenter will introduce a 3D in vitro model designed to test BBB permeability to therapeautic antibodies. The final presentation will discuss novel multiscale in silico models for unraveling complex cellular dynamics of BBB development in a computational neurovascular unit (cNVU) system, whereby toxicity pathways interact with fundamental morphoregulatory signaling (e.g., Wnt, Shh, Delta/Notch) during windows of vulnerability to developmental neurotoxins. The symposium will conclude with a panel discussion and Q&A session moderated by Dr. Slikker.
Integration of NAMs in Risk Assessment: The Read-Across Approach of the EU-ToxRisk Project

S. E. Escher, Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany. Sponsor: M. Leist

The integration of NAMs, new approach methodologies into a comprehensive risk assessment framework is challenging, in particular for complex endpoints such as repeated dose or reproductive toxicity. In EUToXRisk, scientists with different types of expertise develop integrated approaches to testing and assessment (IATAs) for these two endpoints. We believe that the in vitro and ex vivo models selected from the EUToXRisk toolbox will provide a better understanding of adverse outcome pathways moving human toxicology towards mechanistic risk assessment. In this talk, we present the concept and outcomes of the read-across case studies from the EUToXRisk programme. We report on the recent results and limitations of integrating cellular and molecular physiology, system biology and high-content technologies (omics, HCl) using modelling to uncover the causal relationships with apical findings arising from traditional in vivo. We will set a focus on the analysis and comparison of transcriptomic data within and between the case study compounds. This data integration will be illustrated by one case study based on structural analogues and one case study in which structurally diverse compounds share a common mode of action. In both case studies the IATA will predict the hazard of the target compound(s) after repeated low dose exposure. Besides qualitative hazard assessment EUToXRisk aims to derive threshold values, below which compounds are considered to be safe. Therefore, we will show the application of the newly developed ‘in vitro distribution model’ that estimates the intracellular concentration of compounds in the tested cell or tissue model. The intracellular concentration is the starting point to model the oral equivalent dose with IVIVE-PBPK models, which are also under development in EUToXRisk.

International Acceptance of Read-Across Based on NAMs

H. Kamp, BASF SE, Ludwigshafen, Germany.

Hazard assessment of substances such as (agro-)chemicals and pharmaceuticals requires a significant amount of toxicological testing, which is currently based to a large extent on animal studies, and often very costly as well as additionally an ethical challenge. One way to overcome animal testing is the use of alternative methods (3R methods) or nowadays so-called new approach methodologies (NAMs). On the other hand, for the safety assessment of chemicals, a powerful tool to avoid animal testing is read-across, which is basically a comparison between two similar chemicals and the usage of data from one chemical (called source compound) to assess the corresponding other chemical (target compound). This concept is of particular importance under the European chemicals policy REACH. According to current guidance given by regulatory agencies (e.g., the Read-Across Assessment Framework, RAAF, ECHA 2015), similarity is basically defined as structural (and physico-chemical) similarity. The similarity, however, can be substantiated by kinetic or toxicological information. Current experience with read-across cases that have been submitted to regulatory authorities show, however, that many read-across cases have so far not been accepted. Besides the fact that in some cases the structural similarity was questioned, many of the rejected cases have been insufficient in proving toxicokinetic or toxicodynamic similarity to an acceptable extent. Reasons for non-acceptance as well as best practices will be shown using rejected as well as accepted read-across cases from industry. These will comprise toxicokinetic as well as toxicodynamic similarities. From this, recommendations for robust read-across cases will be presented, that can not only help for the development of a good read-across for chemicals, but also enable the regulators for the assessment of, e.g., environmental metabolites of pesticides or impurities in pharmaceutical active ingredients. It goes without saying that these approaches provide key measures for the safety assessment of cosmetics, for which animal testing is completely banned in some areas, e.g., Europe.

Automated Read-Across and Good Practices

T. Hartung, Johns Hopkins University Center for Alternatives to Animal Testing (CAAT), Baltimore, MD.

Modern information technologies have made big data available in safety sciences. The challenge is now to make big sense of these data. Read-across is gaining momentum with increasing data availability and consensus on how to process and report it. It is predominantly applied to in vivo test data as a gap-filling approach but can similarly complement other incomplete data sets. Big data are first of all repositories for finding similar substances and ensure that the available data is fully exploited. Substance similarity analysis was used to determine clustering of substances with similar hazard labels. The European Chemical Agency warehouses the largest dataset of in vivo and in vitro toxicity tests. This data was extracted using linguistic search engines into a structured, machine readable and searchable database. An expanded database was built with 70 million structures of which 30% have biological/chemical properties and for about 20k mostly animal-study-based classification data. The latter were predicted for the six hazards by a novel read-across SAR. Leave-one-out cross-validation for models built on 58,219 known binary chemical health hazards (6 hazards, 43,996 positive, 14,223 negative chemicals) found in 42,479 cases sufficiently similar chemicals allowing predictions for 87% of cases. As structural neighbors correlate with certainty of prediction, any estimate is associated with an uncertainty measure. Here, a new web-based tool REACHacross developed with Underwriters Laboratories is presented, which aims to support and automate structure-based read-across for acute oral and dermal toxicity, eye and skin irritation, mutagenicity and skin sensitization. Together with the currently developed Good Read-Across Practice guidance, this will facilitate the application of high quality read-across. Next, a data fusion approach was implemented building a single model for nine hazards using not only the information within one hazard but all labeling categories including chemico-physical information. The new hazards are acute inhalation, acute and chronic aquatic toxicity. Data fusion boosted accuracy to an extent that no minimum similarity was required, i.e. estimates were done for all chemicals with BAC 88%. Overall, with average 91% sensitivity these predictions outperform repeat animal studies.

A View across the Atlantic: Synopsis and Opportunities

S. Fitzpatrick, US FDA, College Park, MD.

For many of the chemicals found in food, cosmetics, and dietary supplements, CFSAN/US FDA has limited toxicological data and no legal authority to request these data from the sponsors of the compounds. CFSAN/US FDA needs new toxicological approaches that can generate needed information to assure the safe use of these compounds for consumers. Critical to CFSAN/US FDA’s ability to reach sound decisions and to retain the public’s trust are high-quality data and a scientific review process that is thorough, unbiased, and transparent. Regulators must also determine how much evidence is sufficient to determine that a new tool is qualified to make safety decisions that potentially affect millions of consumers. Read-across has the potential to be a useful tool for US FDA because it uses the already available data of a data-rich substance to evaluate the potential toxicity of a data-poor substance which is considered similar enough to the source substance to use the same data as a basis for the safety assessment. In order to learn more about this tool, US FDA and CAAT co-sponsored two workshops on Good Read-Across Practices. The first was in Brussels on February 26, 2016. The second was at US FDA’s Wiley Campus in College Park on March 1, 2016. US FDA and CAAT also sponsored an additional Read Across Workshop in conjunction with the 2017 SOT Meeting. Currently CFSAN/US FDA has partnered with Underwriters Laboratories to assess whether its Reach-Across Program can be a useful predictive tool for assessing the toxicity of chemicals of concern for US FDA.

A Herculean Switch? Rethinking Chemical Carcinogenicity Assessment

S. Papininen, Corteva Agriscience, Indianapolis, IN.

The two-year rodent cancer bioassay has been the standard regulatory requirement to predict carcinogenicity following human exposure to chemicals, including industrial chemicals and agrochemicals, food additives, pharmaceuticals, and environmental pollutants. Decades of experience conducting two-year rodent cancer bioassays have not found a better way to predict human cancer pathogenesis. There have been questions about the scientific limitations and usefulness of the bioassay, especially in light of the resources, time, and animal use associated with the test. Moreover, further questions on the relevance of these data to assess human health risk arise due to the use of very high dose levels in these studies, which are several orders of magnitude higher than real-world human exposures. To address concerns over the human relevance of the assay, a shift in thinking process has paved a path to many cross-sector efforts to rethink carcinogenicity assessment. The first presentation will provide a historical perspective on development and adoption of the rodent cancer bioassay and the efforts to moderate its use in 2007. The second presentation will focus on alternative testing methods to carcinogenicity assessment to improve the efficiency of chemical risk assessment and produce data that are more relevant to protecting human health. The third presenter will share a global perspective on efforts to develop integrated approaches to testing and assessment and adverse outcome pathways to better understand the mech-
Anisms that lead to carcinogenicity in humans and that can be used to design alternatives to the currently used bioassay. The fourth presentation will describe a decision tree and criteria developed within the agrochemical sector and share the results of how they have been used to retrospectively analyze the need for the cancer bioassay in regulatory decision-making. The final presentation will share the US EPA Office of Pesticide Programs’ perspective on alternative methods of testing, including a weight of evidence approach incorporating all the relevant data available including exposure, and will share their vision going forward on waiver criteria for rodent cancer bioassays. Overall, the session will include presentations from scientists across different sectors and enable a panel discussion between the speakers and audience on the value of the cancer bioassay, which has been used as a standard for decades by global regulatory bodies despite being resource, time, and animal intensive. Finally, the session will discuss alternative approaches that could be used to assess carcinogenicity, identifying remaining gaps and shedding light on ongoing global efforts on this subject.

**1632 History of the Rodent Cancer Bioassay in Chemical Risk Assessments and What Did We Learn?**

A. Hayes, University of South Florida, and Michigan State University Institute of Integrated Toxicology, Tampa, FL.

For more than half a century, the rodent cancer bioassay has been used to identify chemicals that may have the potential to cause cancer in humans and has long been used by worldwide regulatory authorities for assessing carcinogenicity and managing risk. Although the cancer bioassay has been embedded in institutional practice for decades, it has long been the subject of controversy. Numerous workshops, retrospective analyses, and review articles have identified issues surrounding the bioassay, and many have questioned the predictability, utility, and even the necessity of this assay when other more appropriate means of achieving information to determine the cancer potential in humans and inform risk management decisions are available. While there remain strongly held opinions on the bioassay, the 21st century trend in toxicology is to promote hypothesis-driven, tier-based testing that draws on a broad array of information from several sources, including predictions from computational and molecular approaches. While many papers in the scientific literature discuss potential improved alternatives to the rodent cancer bioassay, there has not been a concerted effort to bring disparate views together with the objective of identifying a definitive alternative approach that could be implemented today. This talk will discuss when and why the bioassay was developed, what we have since learned about its relevance, and steps that have been taken to modernize the carcinogenicity testing approach.

**1633 Alternative Testing Paradigms to Assess Chemical and Pharmaceutical Carcinogenicity**

C. Wood, Boehringer Ingelheim Pharmaceuticals, Inc., Durham, NC. Sponsor: S. Papineni

Current interest in modifying toxicity testing requirements is driven by needs to improve the efficacy and efficiency of chemical risk assessment, incorporate new higher-throughput types of data into regulatory review, and reduce the number of animals used in testing. An important focus of these efforts is the long-term rodent carcinogenicity study, which has been a standard testing requirement for registration of food-use agrochemicals and other high-priority chemicals for more than 30 years. Recently, a proposal to change the international guidance for carcinogenicity testing of pharmaceuticals was submitted to the International Conference on Harmonisation. This change in the handling of data from non-carcinogenicity studies that would justify whether or not a long-term rodent bioassay would impact the overall cancer risk assessment of a compound. This presentation will address alternative methods for cancer risk assessment and whether a similar type of waiver system could be applied to environmental chemicals. Proposed approaches include paradigm testing schemes, empirical models, and biomarker panels based on specific pathways of chemical carcinogenesis. Recent case studies investigating the use of subchronic histopathology indicators, hormonal activity, quantitative genomic biomarkers, and in vitro bioactivity screens to predict carcinogenicity (or lack of it) in the rodent test species will be presented. New integrative testing paradigms that incorporate predictive accuracy of short-term tests with both human relevance and exposure considerations.

**1634 Moving Forward in Carcinogenicity Assessment: An International Perspective**

R. Corvi, EURL ECVAM, Ispra, Italy. Sponsor: S. Papineni

Several opportunities are available to exploit recent advances in approaches to move away from the two-year cancer bioassay in rodents. Initiatives, including data- or knowledge-driven approaches and those that focus on a disease outcome to establish chemical screening and testing methods, were discussed at a recent workshop organised by the EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) and the European Society of Toxicology In Vitro (ESTIV). Non-genotoxic carcinogens are of particular concern, because they contribute to an increased cancer risk and a variety of mechanisms that are not yet directly assessed by international regulatory approaches. Under the auspices of the Organisation for Economic Cooperation and Development, a working group is currently developing an integrated approach to testing and assessment to address non-genotoxic carcinogenicity. The assay by targeting cancer on their hallmark of cancer that address early to mid and later key events with increasing associations to adversity (e.g., cell proliferation, immunosuppression, cytoskeleton modifications, and angiogenesis). In addition, inspired by the pharmaceutical proposal for refining the criteria for when carcinogenicity testing may or may not be warranted, the European Partnership for Alternative Approaches to Animal Testing (EPAA) is carrying out a project to evaluate whether a similar approach is also applicable to the carcinogenicity assessment of agrochemicals. The aim is to provide evidence that data from 3-month repeated dose toxicity studies together with mode of action data can, in some cases, be leveraged to predict human relevant carcinogenic potential of agrochemicals. Despite a seemingly diverse range of strategic developments across various initiatives and sectors, a number of common critical elements are emerging. For example, there is a clear need to incorporate mechanistic reasoning into carcinogenicity hazard assessment, and to integrate all available information, including data from epidemiology, traditional and alternative toxicology tests, and from novel data streams (e.g., omics, high-throughput technologies). Such cross-sectorial knowledge sharing and harmonization will build confidence in new approach methods and ultimately lead to more effective and efficient safety assessment.

**1635 A Weight of Evidence Approach to Assess Carcinogenicity Potential and Retrospective Analysis of Agrochemicals**

S. Papineni, Corteva Agriscience, Indianapolis, IN.

The two-year cancer bioassay in rodents has been considered the gold standard to assess carcinogenicity across multiple sectors. Following decades of experience conducting the bioassay, the value of this study has become the subject of debate in the scientific community owing to its significant use of animals and limitations, including the relevance of findings to humans. Advancements in technology and our understanding of key events in the development of carcinogenesis have spurred multiple efforts across sectors to make this study a conditional requirement instead of a default study. There is an effort ongoing within the agrochemical sector leveraging the knowledge gained from other sectors, especially the pharmaceutical sector, which is assessing the feasibility of revising the current ICH S1 guideline on rodent carcinogenicity testing. This presentation will provide an update on an ongoing collaborative effort among the agrochemical industry, the animal protection sector, and regulatory agencies to develop a decision tree and criteria to assess the need for a two-year cancer bioassay for agrochemicals. This approach considers knowledge of intended targets, genetic toxicology study results, histopathological evaluation of repeat dose studies, and other information. This presentation will present the results of retrospective analysis carried out to validate the usefulness of the criteria to assess the need for a cancer bioassay.

**1636 Commitment at the US EPA/OPP to Reduce and Replace Animal Use: Looking at Carcinogenicity and Beyond**


The US EPA’s Office of Pesticide Programs (OPP) is committed to implementing strategies and programs to improve risk assessments while reducing animal use in pesticide testing. For example, OPP has developed and implemented a transparent, stepwise process for evaluating and implementing alternative methods of testing for acute oral, dermal, and inhalation toxicity, as well as eye and skin irritation and skin sensitization. Furthermore, OPP has published guidance on waivers for acute and subchronic neurotoxicity, subchronic inhalation, subchronic dermal, and immunotoxicity studies.
Irritation tests by adopting the ISO 10993-23 standard for medical device irritation testing. Consequently, the test protocol will now be adapted and included in a new ISO 10993-23 standard for medical device irritation testing.

W 1638 Time for a Change: The ISO 10993 Standards and Irritation Testing of Medical Devices
K. Coleman, Medtronic plc, Minneapolis, MN.

The ISO 10993 standards have governed biological evaluation of medical devices for a quarter century. These standards address animal welfare, materials characterization, toxicity testing, risk assessment and other issues surrounding biocompatibility assessment of medical devices. Skin irritation is a biological effect that must be considered for virtually every medical device regarding biocompatibility assessment of medical devices. OECD Test Guideline 439 describes the use of reconstructed human epidermis (RHE) as an in vitro test system for classification of skin irritation by neat chemicals. This test, which was developed in the 1940s, has been used worldwide for decades. In the 1980s a modified version was added in the Tripartite Agreement for Medical Device Biocompatibility, and in the 1990s it was included in the ISO 10993-10 standard on irritation and sensitization testing. The test is considered a gold standard for medical device irritation assessment; however, it has some shortcomings. This presentation will provide a brief overview of the ISO 10993 standards, discuss the biology of skin irritation, review the Draize rabbit skin irritation test, and tell the backstory of the search for a replacement including the completion of an in vitro irritation feasibility study.

W 1639 Modification and Use of Reconstructed Human Epidermis (RHE) Models
A. Turley, Nelson Laboratories LLC, Salt Lake City, UT.

The ISO 10993 standards have governed biological evaluation of medical devices for a quarter century. These standards address animal welfare, materials characterization, toxicity testing, risk assessment and other issues surrounding biocompatibility assessment of medical devices. Skin irritation is a biological effect that must be considered for virtually every medical device regarding biocompatibility assessment of medical devices. OECD Test Guideline 439 describes the use of reconstructed human epidermis (RHE) as an in vitro test system for classification of skin irritation by neat chemicals. This test, which was developed in the 1940s, has been used worldwide for decades. In the 1980s a modified version was added in the Tripartite Agreement for Medical Device Biocompatibility, and in the 1990s it was included in the ISO 10993-10 standard on irritation and sensitization testing. The test is considered a gold standard for medical device irritation assessment; however, it has some shortcomings. This presentation will provide a brief overview of the ISO 10993 standards, discuss the biology of skin irritation, review the Draize rabbit skin irritation test, and tell the backstory of the search for a replacement including the completion of an in vitro irritation feasibility study.

W 1640 Creating Irritant-Infused Polymers for Extraction and Irritation Analysis
B. Rollins, ConvaTec, Greensboro, NC.

After years of tissue development, the final hurdle for the round robin validation study using reconstructed human epidermis (RHE) tissues for medical devices was the development of extractable controls. Previous RHE/medical device feasibility studies were completed without irritant polymers, so for the round robin study, positive control materials were created by infusing polymers with known irritants. In order to successfully incorporate hydrophilic and hydrophobic irritant chemicals, a variety of medical device polymers were investigated. The final four polymer-releasing irritants were chosen based on their irritation potential, octanol/water partition coefficient, miscibility with polymer matrices, and use in the medical industry. Verification was performed through extraction and chemical analysis, along with RHE tissue irritation testing prior to their inclusion in the round robin validation study. In this presentation, we will discuss the development of the four novel compounds through unique mixing and curing methods, the challenges encountered during development including the various irritants, concentrations, mixing, and curing processes executed to determine the amount needed to elicit an appropriate irritant response without destabilizing the polymers, and the variety of irritants that were trialed.

W 1641 The Application of Reconstructed Human Epidermis (RHE) Models as In Vitro Skin Irritation Tests for Detection of Irritant Activity in Medical Device Extracts
W. De Jong, National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands. Sponsor: A. Turley

Assessment of skin irritation is an essential component of the safety evaluation of medical devices. Testing the irritant capacity of medical device extracts is currently performed by either topical or intradermal injection in rabbits according to ISO 10993-10 as part of the ISO 10993 series for the biological evaluation of medical devices. OECD Test Guideline 439 describes the use of reconstructed human epidermis (RHE) as an in vitro test system for classification of skin irritation by neat chemicals. An international round robin study was conducted to evaluate the RHE method for determination of skin irritant potential of medical device extracts. Four irritant polymers and three non-irritant controls were obtained or developed that had demonstrated their suitability to act as positive or negative test samples. The RHE tissues (EpiDerm™ and SkinEthic™ RHE) were dosed with 100 µL aliquots of either saline or sesame oil extract. Incubation times were 18 h (EpiDerm™) and 24 h (SkinEthic™ RHE). Cell viability reduction > 50% was indicative of skin irritation. Both the EpiDerm™ and SkinEthic™ RHE tissues were able to correctly identify virtually all of the irritant polymer samples either in the saline or sesame oil extract. Our results indicate that RHE tissue models can detect the presence of strong skin irritants at low levels in dilute medical device polymer extracts. Therefore, these models may be suitable replacements for the rabbit skin irritation test to support the biological evaluation of medical devices. Consequently, the test protocol will now be adapted and included in a new ISO 10993-23 standard for medical device irritation testing.

W 1642 Taking Full Advantage of the MDDTs: Using Tools for Regulatory Risk Management in the Medical Device Development Process
H. Scharen. US FDA, Silver Spring, MD. Sponsor: A. Turley

The medical device development tools (MDDTs) process provides a new route for interested parties to present novel testing methods to the US FDA. Throughout the medical device development process, MDDTs can be used in the evaluation of medical devices; these tools help manufacturers understand the safety, effectiveness, and performance of medical devices. Qualified MDDTs provide assurance that the measurement techniques produce scientifically meaningful and valid results within the Context of Use (COU). The COU describes the specific way the MDDT should be used in device evaluation and regulatory submissions including the output/meter from the tool. Tool categories include biomarker tests, clinical outcome assessments (such as clinician reported rating scales), and non-clinical assessment models (such as in vitro models to replace animal tests). This presentation will provide an
overview of when the MDDT process is appropriate, a review of the overall process for qualifying a MDDT, and how the MDDTs apply to testing procedures. The qualification process for in vitro irritation testing will be used as a case study with attention given to the practical questions of cost and protocol ownership.

**1643 Applying Systems Biology Approaches to Understand the Joint Action of Chemical and Nonchemical Stressors**

**C. Rider, NIEHS, Research Triangle Park, NC.**

Toxicology has evolved from a strictly observational science to a more predictive one that relies on knowledge of stressors (chemical or nonchemical) and the biological systems with which those stressors interact to identify health hazards. Furthermore, the introduction of the exposome concept brought into sharp focus the fact that humans are routinely exposed to a great number of chemical and nonchemical stressors over the course of a lifetime. Chemical stressors can arise from everyday use of personal care products and consumer products, occupational exposures, and exposure to pollutants through contaminated air, water, and/or food. Nonchemical stressors include physical stressors (e.g., heat and cold, radiofrequency radiation, biological agents such as allergens and viruses), and psychosocial stressors (e.g., noise, exposure to violence), which involve both exposure and perception. The traditional reductionist view reflected in toxicology by the study of one chemical at a time falls short of the promise of high-throughput technologies, a focus on biological pathway disruption, and acknowledgement that real-world exposures are complex. In response to the evolution of our thinking in toxicology and exposure science, a rational approach to address the challenges of mixtures has emerged. This conceptual framework incorporates systems biology and mixture toxicology. Mixtures approaches take a more mechanistic approach that links independent independent groups have developed projects or case studies to work through this conceptual framework. Examples of endpoints include disruption of male reproductive tract development, atherosclerosis, stasis of, and cancer. Although these efforts are in various stages of development, a detailed review of the available programs will provide opportunities to identify challenges, key knowledge gaps, share information, and foster collaboration and cooperation to move the field forward. Case studies, such as the ones presented here, will inform cumulative risk assessment by providing a path forward for determining which stressors to include and how we might move away from a chemical-centric perspective to one focused on the diseases that are of greatest concern to public health.

**1644 Understanding Biological Pathways across Toxicological Tools**

**J. E. Rager, University of North Carolina at Chapel Hill, Chapel Hill, NC.**

Research surrounding systems-level effects of chemical and nonchemical stressors has expanded over recent years, alongside the increasing feasibility of multi-target and -omic-based investigations. Databases relevant to systems biology have also grown, serving as central repositories for toxicological response information that can be leveraged to better understand molecular events resulting from multiple stressors. What remains underdeveloped are methods to effectively relate such data to individual disease phenotypes. Recent progress has been made through the implementation of organizational schemes, including Adverse Outcome Pathways and the Ten Key Characteristics of Carcinogens. These recent advances will be discussed alongside examples of how chemical-induced biological pathway perturbations have been related to disease outcomes, while highlighting the current data gaps and limitations in tools available to understand systems-level effects of chemical and nonchemical stressors.

**1645 Mixtures That Target Male Reproductive Tract Development**

**J. M. Conley, US EPA/ORD, Research Triangle Park, NC.**

Two of the most commonly occurring human birth defects are hypospadias of the phallus and cryptorchidism of one or both testes. Further, reductions in adult sperm counts have been reported in multiple countries. Increases in the prevalence rates of these disorders over the past several decades have been reported and exposure to environmental chemicals has been suggested as a causal factor. A variety of environmental chemicals that operate via a range of molecular mechanisms have been shown to produce male reproductive tract disorders following in utero exposures in laboratory animal experiments. For example, androgen receptor (AR) antagonists (such as vinclozolin) and some phthalates, which decrease testicular testosterone but do not interfere with AR activity, have both been shown to elicit dose-dependent increases in male reproductive tract malformations. During the last 10-15 years we have used a mixtures approach to analyze these chemical-induced mechanisms of male reproductive tract disruption into an Adverse Outcome Pathway (AOP) network. The AOP network converges on adverse outcomes of the developing male reproductive tract and serves as a conceptual model for the design and evaluation of complex mixtures studies. An underlying hypothesis of these mixtures studies is that although the chemicals target different molecular initiating events, their convergence at critical key events will ultimately lead to dose additive adverse effects when exposure occurs as a complex mixture. Results indicate that, despite acting through disparate molecular mechanisms, chemicals impacting male reproductive development act cumulatively to produce adverse effects similar to those observed in human populations. Moving forward, AOP networks provide a mechanistically-based framework for grouping chemicals, addressing mixture hazards, and in conducting cumulative risk assessments. Abstract does not necessarily reflect US EPA policy.

**1646 A Model Disease to Determine the Interaction of Chemical and Nonchemical Stressors**

**D. J. Carlin, NIEHS, Research Triangle Park, NC.**

A critical research area which requires further exploration is the biological mechanisms and effects of exposure to both environmental chemicals (e.g., air pollution, PAHs, metals) and non-chemical stressors (e.g., psychosocial, lifestyle, quality-of-life, poor nutrition, physical stressors) over time and the roles they may play in modifying the development of disease. Recently, the National Institute of Environmental Health Sciences (NIEHS) and the National Heart, Lung, and Blood Institute (NHLBI) held a workshop that brought together experts to discuss the state-of-the-science pertaining to underlying biological pathways associated with, when combined, chemical and non-chemical stressors in relation to atherosclerosis (ATH). ATH 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. This presentation will discuss utilization of the Adverse Outcome Pathway (AOP) framework to evaluate this disease and its mechanisms as a novel approach to combine information from studies to elucidate knowledge of biological pathways, account for species differences or similarities, incorporate genetic and lifestyle susceptibility, and identify research needs. Specifically, discussion will focus on how disparate stressors (e.g., environmental chemicals and non-chemical stressors) may interact together in order to result in ATH. Approaches and research needs include: characterizing qualitative and quantitative impacts of exposure to the combination of chemical and non-chemical stressors, developing new models and methods for assessing the toxicity of multiple co-occurring environmental hazards, and analyzing the effects associated with susceptible and vulnerable populations. This case study presents the use of a systems biology and AOP network approach for identifying factors that induce atherosclerosis, and may serve as a model how to determine the role that chemical and non-chemical stressors play in the development of other diseases.

**1647 The EuroMix Project on Mixtures That Target Steatosis**

**A. Lampen, BfR, Berlin, Germany. Sponsor: C. Rider**

EuroMix is a Horizon 2020 EU project which aims to develop an adverse outcome pathway (AOP) and quantitative test strategy (bioassay toolbox) regarding combined mixture effects of food relevant residues and contaminants. The EuroMix approach includes prioritization of chemicals based on in silico predictions and bioassays, followed by establishment of quantitative exposure assessment groups based on mode of action and/or adverse outcomes. As an example, an in vitro bioassay toolbox for measuring liver steatosis according to the concept of AOPs was developed by including key steps of the AOP using a
human liver cell model. A toolbox comprising reporter gene assays, low density arrays, qRT-PCR, AdipoRed assay, high content cell imaging, LC-MS/MS and GC-FID, as well as Seahorse XS Cell Mito Stress test was used to analyze nuclear receptor activation, gene and protein expression, lipid accumulation and mitochondrial disruption in human HepaRG cells. Different food relevant compound mixtures have been tested and an in vivo proof of concept experiment was performed. As an example, the pesticides thiabendazole and clothianidin sharing a similar or dissimilar mode of action (MoA) regarding liver steatosis (mainly PXR mediated vs. nuclear receptor independent) were successfully shown to induce steatosis in vitro. Analysis of equipotent mixtures of these compounds using dose response (BMD) modelling revealed dose addition effects of either chemicals with similar or dissimilar MoA. The results demonstrate the suitability of the in vitro toolbox for liver steatosis to assess mixtures with a similar or dissimilar MoA. In the next step the bioassay toolbox was focused to a smaller test set reflecting the steatosis AOP in regard to developing a fast and high throughput approach for testing mixtures. Finally, an integrative proof of concept experiment starting with in silico predictions of steatosis relevant chemicals (form QSAR and molecular docking of relevant nuclear receptors) and ending with the established toolbox testing of single compounds and mixtures will be performed.

1648 Converging on Cancer with Systems Toxicology
C. V. Rider. NIEHS/NTP, Research Triangle Park, NC.

The Hallmarks of Cancer described by Hanahan and Weinberg represent the biological pathways that are critical in cancer development. The ten Hallmarks are not discrete events, but interrelated cellular processes that are perturbed during cancerization. The Halifax Project used the Hallmarks of Cancer as a starting place to develop a hypothesis for the effects of environmental mixtures on cancer development. Through this effort, cancer biologists and environmental scientists were brought together to discuss the role of chemical mixtures in cancer development and progression. They hypothesized that environmental chemicals present below levels of concern (based on their individual dose-response data) will contribute to the development of cancer by acting on the 'Hallmark' pathways. The hypothesis was nominated by the Environmental Working Group to the National Toxicology Program to stimulate research on the role of environmental mixtures in cancer. This presentation will highlight the conceptual development of a testing program to address the convergence of environmental chemicals on the network of pathways involved in cancer development. For example, the angiogenesis Hallmark will be discussed in terms of environmental chemicals that have been implicated at inducing angiogenesis and in vitro screening tools that could be used to evaluate candidate chemicals for inclusion in the program. The application of in silico approaches to combine multiple pathways and predict the joint effects of chemicals that target the Hallmark pathways will be discussed.

1649 NextGen Renal Proximal Tubule Toxicity Screening: Novel Cellular Model and Complex Culture Platforms
S. Lu. Pfizer, Inc., San Diego, CA.

Active renal secretion in the proximal tubules is a major drug elimination route, making the kidney susceptible to drug-induced injury. High blood flow to the kidneys significantly contributes to exposure to potential nephrotoxins that enter the cells mostly basolaterally via organic anion and organic cation transporters or apically via reabsorption processes. Many drugs associated with proximal tubule damage are polar, such as acyclovir (cLogP -2.2) and cidofovir (cLogP -2.0), exhibiting poor passive permeability, and hence require active transporters or receptors. To investigate the nephrotoxic potential of lead compounds, in vitro systems should emulate the renal physiologic environment, including functional transport machinery. Cell lines or primary cells traditionally used in 2D kidney toxicity screening lack the appropriate transporter expression, in vivo structure, and function and are unable to predict preclinical/clinical kidney toxicity. Recent biotechnological developments provide more sophisticated and promising models, including 3D culture platforms and reprogrammed renal tubular epithelial cells, which could be utilized to create a more physiologically relevant platform with the potential to improve the prediction value for proximal tubule toxicity screening. The session will provide a general overview of these state-of-the-art biotechnological advances to facilitate the discussion about the path forward for in vitro kidney toxicity screening with high reliability and mechanistic insight.

1650 Early Screening for Nephrotoxicity Employing Transporter Overexpression Cell Lines
S. Lu. Pfizer, Inc., San Diego, CA.

Nephrotoxicity due to drugs and environmental chemicals accounts for significant morbidity and mortality during drug development. As an example, the nephrotoxicity associated with proximal tubule toxicity is hydrophilic — suggesting that active uptake via transporters drives the cell accumulation for such low permeable drugs. However, cells commonly used in in vitro toxicity studies lack transporter expression which contributes to false negative results. Here we used OAT1, OAT3 and OCT2 over-expressing HEK293 cells to improve assay sensitivity for nephrotoxic compounds. This result correlates with the compound uptake data. In addition inhibition of OAT1 and OAT3 (probenecid) and OCT2 (quinidine) reduced the toxicity associated with test compounds indicating that the increased toxicity is associated with increased cell accumulation. These results demonstrate the utility of using transporter over expression cell line to help understand the mechanism of toxicity and potential use as a screening tool.

1651 Directly Reprogrammed Induced Renal Tubular Cells (iREC) for Renal Toxicity Testing
S. Lienkamp. University Medical Center Freiburg, Freiburg, Germany. Sponsor: S. Lu

Direct reprogramming by forced expression of transcription factors can generate desired cell types from differentiated cells without the prior induction of pluripotency. We recently identified four transcription factors that facilitate the direct conversion of mouse and human fibroblasts into induced renal tubular epithelial cells (iRECs). These cells exhibit epithelial features, a global gene expression profile resembling their native counterparts, and functional properties of differentiated renal tubule cells, such as active endocytosis and tubule formation in organoids and decellularized ECM scaffolds. While directly reprogrammed tubule cells offer a potential road to regenerative approaches, a more tangible goal is their use in disease modeling and toxicity screening. iRECs are sensitive to clinically used nephrotoxins and respond by expression of renal injury molecules. For example, treatment with cisplatin changes the metabolic profile of iRECs similar to that of in vivo models. A toxicity induced metabolic signature found in the exometabolome of iRECs was not observed in fibroblasts or other renal epithelial cells (IMCD-3). Therefore, direct reprogramming may be an attractive in vitro strategy to advance patient specific nephrotoxicity testing.

1652 Challenge Accepted: Update on NC3R NephroTube Challenge
M. Wilmer. Radboudumc, Nijmegen, Netherlands. Sponsor: S. Lu

The general aim of the NephroTube Challenge is to develop a multi-compartmental microfluidic device that models renal tubular injury observed in nephrotoxicity. This demands a triad of 1) a human renal cell line with intact transporter function and metabolic activity, 2) sophisticated microfluidics compatible with high-throughput analysis, and 3) high level expertise in toxicology. Developing and implementing of such device has the potential to reduce animal experimentation and improve predictivity of drug-induced kidney injury during drug development. During the NC3Rs CRACK-IT NephroTube Challenge, human immortalized proximal tubule epithelial cell lines (CiPTEC and RPTEC) cultured in a multi-compartmental microfluidic tier-plate (Organato*P) were evaluated as a high-throughput screening tool to assess drug-induced kidney injury. Our kidney-on-a-chip device was exposed to twelve compounds known for their nephrotoxic potential (including cisplatin, tenofovir, tobramycin and cyclosporin A) for 24 and 48h. The format allowed multiple read-outs for toxicity, including cell viability, LDH, NAG, miRNA secretion, gene expression profiling, drug transport interactions and assessment of barrier integrity. Inter-laboratory variation was limited and robustness of the cell-based assays could be demonstrated. Together, the microfluidic tier-plate Organato*P seeded with CiPTEC or RPTEC provides a platform for high-throughput screening which will allow the biomarker discovery to analysis upon exposure to drugs of different classes. Further validation using chemical compound libraries and implementation in drug development is required to demonstrate the value of such models to reduce animal experiments and improve drug safety.
Our lab has developed a 3D model of the human kidney proximal tubule as an ex vivo microphysiological system (MPS) for preclinical toxicity testing. Polymyxins are potent antibiotics but their use is restricted because of nephrotoxicity. To mitigate nephrotoxicity, structural analogues of polymyxins are being developed. Using our MPS, we assessed the safety of polymyxin B and the analogues NAB739 and 741. Following polymyxin B treatment, the US FDA qualified urinary biomarker of acute kidney injury, kidney injury molecule-1, as well as a panel of injury-associated microRNAs, was significantly increased in device effluents. Transcriptional profiling identified cholesterol biosynthesis as the primary pathway induced by polymyxin B. In contrast, we observed minimal changes in gene expression and no significant upregulation of urinary biomarkers in response to the NABs. Our findings demonstrate the improved safety of NAB739 and 741, reveal cholesterol biosynthesis as a novel pathway affected by polymyxin B, and support the use of microphysiological models for safety assessment.

Cannabinoid (CBD) containing products are commercially available without prescription for anxiety, inflammation, chronic pain, and psychological ailments. They are available in a plethora of flavors in oral, sublingual, and inhalable forms (e.g., e-cigarettes) without regulation by the US FDA. We assessed the immuno-toxicological effects of CBD oil by hypothesizing that CBD oil containing products induce oxidative stress and inflammation in macrophages, epithelial cells, and fibroblasts. Products containing CBD at different concentrations (100mg, 350mg, and 550mg) were tested for acellular reactive oxygen species (ROS) production by DCFH-DA fluorescent dye. Cellular ROS levels were assessed by celROX green in monocytes. Epithelial cells (Beas 2B), macrophages (U937), and human lung fibroblast cells (HFL-1) were treated with varying CBD concentrations (between 0.0035 mg/mL and 0.14 mg/mL), and the inflammatory mediators, IL-8 and MCP-1, were measured in the conditioned media by ELISA and cytokine arrays. Furthermore, monocytes and epithelial cells were stimulated with LPS along with CBD as individual and combined treatments to test the antioxidant and anti-inflammatory effects of CBD. The inflammatory regulation mechanism was evaluated by measuring the NF-κB activity in monocytes. CBD generated significant dose-dependent acellular and cellular ROS levels. IL-8 was induced with CBD treatment in various cells. However, the MCP-1 levels were decreased with CBD treatment showing anti-inflammatory effects. LPS stimulated cells showed inhibition of NF-κB activity in CBD treated macrophages. Moreover, CBD significantly attenuated IL-8 and MCP-1 cytokine responses in LPS stimulated macrophages. Cytokine array data depicted differential cytokine response due to CBD in different cell types. Commercially available CBD solutions increase ROS production, thus inducing an inflammatory response. However, inflammation potentiated by LPS can be attenuated or augmented by CBD. Therefore, CBD may be used as an anti-inflammatory agent for an ongoing inflammatory response, but should not be used otherwise. Our data suggest that commercially available CBD oil (if inhaled via e-cigarette aerosol) should be regulated, as unnecessary exposure could potentially cause harm to the consumers. Supported by the Toxicology Training Program grant T32-ES007026.
Chemical agents such as industrial chemicals, pesticides, and chemical warfare agents can induce uncontrolled seizure activity (neuronal hyperexcitability). Oxidative stress and a perturbed glutathione (GSH) redox status are implicated as pathogenic factors in the etiology of seizures and epilepsy. However, whether and how cellular GSH redox status modulates neuronal hyperexcitability is unclear. We hypothesized that the modulation of cellular GSH redox status with thiol containing compounds would attenuate neuronal hyperexcitability in vitro and in vivo. 2,3-dimercaptopropanol (DMP), a thiol-containing compound significantly (p<0.001 vs vehicle control) increased intracellular glutathione levels in mixed rat primary cortical cultures at 4 and 24h. Next, we determined if DMP could dampen "seizure-like" activity in vitro induced by 4 Aminopyridine (4AP), a toxin that inhibits potassium channels. In mixed rat primary-neuronal-glial cultures, incubation with 30µM or 100µM DMP for 4h significantly (p<0.01, p<0.0001 vs 1mM 4AP) decreased 4AP-induced neuronal hyperexcitability. Next, we assessed whether DMP can increase intracellular GSH levels in an in vivo model system. To test this, zebrafish larvae were treated with different DMP doses for 4h. DMP significantly (p<0.01 vs vehicle control) increased intracellular GSH levels in a dose-dependent manner in zebrafish larvae. Finally, we tested the ability of DMP to decrease 4AP-induced seizure behavior in zebrafish larvae. Larvae pre-treated with 100µM DMP for 4h exhibited decreased 4AP-induced seizure behavior. Taken together, the data suggest that the thiol-containing compound DMP alters GSH redox status and controls neuronal hyperexcitability both in vitro and in vivo.

Oxidative stress and release of pro-inflammatory mediators are well established signaling events following inhaled chemical exposure; however, our understanding of the distinct cellular and molecular pathways involved in inhalation toxicity remain limited due to studies centered around a single airway cell type (i.e., epithelial cells), and lack of physiologically-relevant studies on diverse cell types beyond the epithelial barrier. Utilizing an in vitro organotypic model that recapitulates the "trans-epithelial" nature of analogous exposures in vivo, we investigated the effect of the model toxicant (diesel exhaust particulates (DEP)) on human bronchial epithelial cell (HBEC) and lung fibroblast (HLF) oxidative stress and pro-inflammatory signaling. We hypothesized that trans-epithelial exposure to DEP drives oxidative stress and pro-inflammatory response through an imbalance of oxidant/antioxidant gene expression and alternative cellular signaling pathway activation within the airway microenvironment. Temporal (2-24 hours) gene expression analysis demonstrated peak HMOX-1 response at 6 hours in HBEC and HLF, while IL-8 peaked at 4 hours in HBEC and 10 hours in HLF, by which time HM0X-1 had declined substantially. Genes involved in glutathione homeostasis and H2O2 signaling (NQO1, TRX1, PTGS2 and GCLM1) were alternatively regulated in response to DEP exposure. Live-cell imaging with a dual fluorescent biosensor for glutathione oxidation and intracellular H2O2 accumulation (roGFP2/Hyper red) showed that treatment with DEP led to an increase in overall oxidation in both cell types, which was attenuated by pre-treatment with the free radical scavenger, N-acetyl-cysteine (NAC). Upregulation of antioxidant response and pro-inflammatory genes in our studies suggested a role for key stress-response pathways (MAPK, NF-kB and Nrf2) in DEP-dependent oxidative stress and pro-inflammatory signaling. The kinetics of MAPK, NF-kB and Nrf2 pathway activation by DEP exposure were determined via time-course (2-24 hours) immunoblotting and densitometry analysis; confirming DEP-mediated and time-dependent phosphorylation of key cellular targets. This study is the first to characterize the dynamics of oxidative stress and pro-inflammatory signaling within the airway microenvironment following trans-epithelial chemical exposure and provides novel insight for the development of therapeutic and/or preventative interventions to reduce adverse effects of inhaled chemical exposure.

Hydroxia induces lung inflammation and injury, which significantly impacts patient morbidity and mortality. Prior studies show protective effects of cytochrome P450 (CYP1A1) enzymes against hyperoxic lung injury. Nrf2 is an important transcriptional regulator of antioxidants and loss of Nrf2 leads to exacerbation of hyperoxic lung injury with reduced antioxidant expression in lung tissue and mortality in mice. Studies to rescue the Nrf2-/- phenotype against oxygen injury are lacking. In this investigation, we tested the hypothesis that hyperoxia induces lung inflammation and inflammation in Nrf2-/- mice compared to wild type (WT), and that this phenotype will be rescued by the free radical scavenger, N-acetyl-cysteine (NAC). Lung Cyp1a1 transcription was elevated with BNF treatment and loss of Nrf2 leads to hyperoxic lung tissue and mortality in mice. Studies to rescue the lung injury phenotype in Nrf2-/- mice suggests that augmented CYP1A expression by BNF may contribute to the beneficial effects. Further studies could lead to the development of BNF and other flavonoids for the prevention/treatment of hyperoxic lung injury.

Oxidative stress and reactive oxygen species (ROS) released during acute inflammatory processes contribute to airway injury in allergic asthma (1). Innate immune cells, including monocytes/macrophages and neutrophils, are key contributors to inflammatory mediators including ROS (1). Monocytes/macrophages are critical in the regulation of tissue damage by reacting to initial oxidative stress (1). Limitations include the lack of understanding of monocytes/macrophages and neutrophils in the development of therapeutic and/or preventative interventions to reduce adverse effects of inhaled chemical exposure. JUUL and vaping pens are emerging electronic nicotine delivery systems (ENDS) in western populations. They have become increasingly popular among youth due to compartmentalized packaging, appealing flavors, and as health supplements. Moreover, these ENDS are also perceived as a healthier alternative to traditional cigarettes, thus have become a public health concern. The health effects caused by JUUL and vape pens are currently unknown. We hypothesized that JUUL and vape pens constituents, primarily the flavoring agents, will induce cytotoxicity, oxidative stress, and inflammation. Acellular reactive oxygen species (ROS) levels generated by various JUUL flavors (cool mint, mango, crème brulee, Virginia tobacco, and cool cucumber) and vaping pens (vitamin B12, sleep, energy, and performax) were evaluated by DCFH-DA fluorescent dye. These flavors were also assessed for their ability to deplete glutathione (GSH) and form oxidized glutathione (GSSG). To evaluate cellular effects due to acute exposure, lung epithelial cells (Beas 29) and macrophages (U937) were exposed to aerosols generated by JUUL and vape pens. Cytotoxicity was determined by measuring the cell viability by (AO/PI) staining; IL-8 cytokine levels were measured in the conditioned media by ELISA to assess the inflammatory response. The reduced ROS levels induced in the control group. JUUL cool mint produced the highest levels of ROS in JUUL peaking at 29 µM H2O2 equivalence. Vitaminvape B-12 produced the highest levels of ROS for vape pens peaking at 121 µM H2O2 equivalence. These flavors had differential effects on GSH levels compared to the control group. These flavors did not significantly affect cell viability, but induced differential IL-8 levels in the exposed cells. Our data show that JUUL and vape pens flavor may trigger a pro-inflammatory response mediated by oxidative stress. These data suggest that JUUL and vape pens are not a risk-free method of vaping and the constit-
In Vitro Exposure of Lung Cells to Electronic-Cigarette Aerosols at the Air-Liquid Interface Is Cytotoxic, Alters Gene Expression and Levels of Reactive Oxygen-Nitrogen Species

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Over 8 million US adults are regular e-cig users. Emerging evidence indicates that e-cig aerosols adversely affect lung function; however, the cellular and molecular mechanisms underlying these effects remain unknown. To examine the effects of e-cig aerosol exposures on lung cells in an in vitro exposure system representative of human pulmonary deposition, confluent human lung epithelial cells (H292), human pulmonary fibroblasts (MRC-5), and mouse embryonic fibroblasts (NIH-3T3) were exposed to butter-flavored (diacetyl) e-cig aerosols at the air-liquid interface (ALI) in a Virtrocell exposure system connected to a third-generation e-cig (SCIREQ). Exposures were conducted following a standard transpulmonary topology profile for 1-hr per day for 1, 3, or 6 consecutive days. Following ALI exposures, cell viability and cytotoxicity were assessed; exogenous nitric oxide (NO), and reactive oxygen species (ROS) levels were measured in the culture media; and gene expression was analyzed by qRT-PCR. E-cig aerosol-exposed H292 cells had significantly reduced numbers of viable cells and increased levels of lactate dehydrogenase (LDH), a marker of cytotoxicity, (>87 and 114%, 3 and 6 days, respectively) compared to air controls. While NO levels were markedly reduced at >63% and 6 days (>92%), extracellular ROS levels increased by >40% at both time-points. A 1-day e-cig exposure supported these results with significantly augmented mRNA levels of HMOX1 (oxidative stress), HPRT1 (hypoxia), h<sub>2</sub>mall (apoptotic), and IL-6 (inflammation). Similarly, e-cig decreased cell viability and increased LDH levels in MRC5 (>18%) and NIH3T3 (>145%) cells at both time-points. At 3 days, e-cig decreased exogenous NO levels (MRC5: >33%; NIH3T3: >85%) and increased the ROS activity (MRC5: 49% and NIH3T3: 113%). There is a time-course relationship where e-cig exposure appears to decrease NO production. Further, human lung epithelial cells may be more sensitive to e-cig aerosols than lung fibroblasts. Overall, long-term exposures to e-cig aerosol may induce detrimental effects in the lungs due to cytotoxicity, enhanced oxidative stress, low levels of NO, and altered expression of key genes associated with oxidative stress, airway remodeling and inflammation.

Target Safety Assessments: Evaluation of the Toxicological Risk of Targeting FRS (Phenylalanyl-tRNA Synthetase) in the Treatment of Malaria

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Phenylalanyl-tRNA synthetase (FRS) is a highly conserved enzyme that catalyzes the ligation of phenylalanine to its cognate transfer tRNA during protein synthesis. Due to its vital role as part of the translational machinery, FRS has been identified as a potential target to treat the malaria parasite. However, since target-related toxicity accounts for >50% of all drug project failures, it is vital to understand the potential unintended consequences of target modulation in non-plasmodium (mammalian) species to assist in the determination of the required plasmodium/human safety ratio. We conducted a comprehensive in silico target safety review to understand the role of FRS in normal physiology as a basis for evaluation of the potential toxicity of FRS inhibitors. Based on published literature, it is clear that eukaryotic cellular harbours two different types of FRS: the heterotetrameric cytosolic alpha (FRSA) and beta forms, and the monocmeric mitochondrial forms. Pathogenic variants in FRSA (encoding the human mitochondrial FRS) have been associated with phenotypes ranging from spastic paraplegia to fatal infantile Alpers encephalopathy. FRS1 knockout mice are homozygous lethal. Heterozygote phenotypes include abnormal bone morphology, decreased bone mineral density, decreased circulating chloride and sodium levels, impaired glucose tolerance and increased total body fat amount. Based on these observations, we predict that potential target organs of toxicity caused by inhibition of FRS could include bone, heart, immune system, kidney, and the reproductive system. Specifically, there may be a risk of abnormal bone development, perturbed glucose metabolism, immunosuppression, nephotoxicity, reduced liver function, myopathy and an increased risk of epilepsy. Based on this toxicological profile, inhibition of host FRS could be a serious limitation; therefore, the specificity and selectivity of compounds will be a key for their success. However, a single genomic copy of mitochondrial FRS is targeted to the parasite mitochondria and is exclusive to malaria parasites within the apicomplexan phyla, hence drug targeting of FRS presents a unique opportunity to potentially target malarial FRS specifically. Nonetheless, it would be sensible to conduct an early rodent investigative study looking at in life effects and potential target organs to help identify whether the risks in an silico analysis has identified actually occur in vivo with inhibitors of FRS.

Efficacy and Preclinical Pharmacokinetics and Toxicology of L-Propargylglycine, a Prototype Inhibitor of Sleep Disordered Breathing (Sleep Apnea)

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More than 25 million Americans demonstrate sleep disordered breathing (SDB). In addition to interfering with normal sleep patterns, SDB has been linked to serious secondary pathologies that include cardiac arrhythmia, hypertension, and stroke. However, no effective drug therapies are available to treat SDB. We have recently reported that hypersensitivity of the carotid body is an important mechanism underlying SDB, and that this carotid body hypersensitivity results from enhanced generation of H<sub>2</sub>S by cystathionine γ-lyase (CSE). In the present studies, we used a well-known inhibitor of CSE, L-propargylglycine (L-PAG), as a prototype agent to investigate the hypothesis that CSE inhibition is an effective means to prevent SDB. L-PAG is a potent inhibitor of SDB in the HO-2-/- knockout mouse model. L-PAG doses ≥ 90 mg/kg completely inhibit SDB. L-PAG is rapidly absorbed after oral dosing and T<sub>max</sub> = 0.25 to 0.5 hr, and oral bioavailability is 100%. Plasma concentrations of L-PAG increase in proportion to dose, and are directly related to efficacy in SDB inhibition: 30% efficacy is seen at plasma levels of ~50 μM (oral dose of 10 mg/kg), and 100% efficacy in SDB inhibition is seen at plasma levels ≥ 500 μM (oral doses ≥ 90 mg/kg). Evidence of gross toxicity was seen only after single dose administration of L-PAG at 750 mg/kg (plasma level of ~4500 μM), providing an efficacy to toxicity ratio of ≥ 8 (toxic dose versus 100% inhibitory dose) to as much as 75 (toxic dose versus 30% inhibitory dose). A 7-day repeat-dose screening toxicity study of L-PAG and its enantiomer D-PAG was performed in C57BL6 mice. At doses of up to 100 mg/kg/day (an L-PAG dose that completely inhibits SDB in HO-2-/- mice), neither L-PAG nor D-PAG had significant effects on survival, clinical signs of toxicity, body weights, hematology, clinical chemistry, or gross pathology at necropsy. L-PAG also had no effect on in vitro hERG activity. However, screening Ames tests of L-PAG and D-PAG in TA98 and TA100 tester strains demonstrated that both agents are mutagenic (both with and without metabolic activation); D-PAG was the more potent mutagen. These data demonstrate that CSE inhibition provides a viable mechanism for prevention and treatment of SDB. However, the mutagenicity of L-PAG makes it unsuitable for further preclinical or clinical development. NHLBI grant SU5H3HL123610.

Optimising Kinase Selectivity for Non-Oncology Indications


The development of kinase inhibitors for non-oncology indications demands a stringent safety profile. Each indication and/or patient population will determine the acceptable tolerability profile. Early focus on selectivity is therefore required and best addressed with a cross-functional approach. Unified kinase profiles and data analysis within and across projects are needed, and enable a better understanding of selectivity requirements and potential associated adverse effects. Tools to correlate the latter to certain off-target kinase hits and pathways involved are extremely valuable to determine a path forward. Although providing an exhaustive list of ‘kinases to avoid’ is not feasible, literature-based analysis of frequent off-target kinase hits is a first step to assess if building selectivity should be considered. Once undesirable kinase hits have been identified, pIC<sub>50</sub>s should be generated to enable ligand based SAR. However, enough datapoints are needed to explore the potential of this QSAR model and enable the prediction of off-target kinase hits for future compounds. The availability of crystal structures of ideally both target and related off-target kinases is another valuable tool to enable selectivity by design. These models are crucial since in vivo toxicity studies should only be performed once sufficient selectivity has been achieved to adequately inform further development of the compounds. The ultimate goal is to enable the development of kinase inhibitors with an acceptable risk-benefit profile for non-oncology indications.
P-glycoprotein (P-gp, MDR-1) is an ATP-dependent efflux transporter that is highly expressed in the gastrointestinal epithelium, liver, pancreas and kidneys, as well as the capillary endothelial cells that establish the blood-brain barrier. P-gp interacts with a diverse array of small molecule xenobiotics, including many drugs. Drugs that are substrates of P-gp are actively expelled from the cell, whereas drugs that are inhibitors of P-gp effectively abrogate the transporter function. Either interaction with P-gp can greatly impact the efficacy, potency, and safety of a co-administered drugs, shifting its rate and maximal concentration of absorption, distribution, metabolism, and eventual excretion (ADME). Assessing a drug’s potency as a non-substrate, substrate or inhibitor of P-gp, and thus its potential liability for inducing downstream drug-drug interactions (DDI), is a critical component of the drug development process mandated by the US FDA. In the current study we validate a new and sensitive cell-based P-gp activity assay and demonstrate its utility in the rapid screening of drug candidates to identify those that interact with P-gp. A small library of drugs was screened, and those presenting incidental activity as a P-gp substrate or inhibitor were classified and ranked by potency. Selected individual P-gp inhibitors were then administered to upcycled human hepatocytes in combination with a dose-regimen of individual second drugs that were also identified to be substrates of P-gp and previously characterized as modulators of CYP gene expression, or as hepatotoxins. In combination, these data reveal the significant impact of P-gp interactors on altering CYP expression profiles and increasing hepatotoxicity liabilities. The in vitro P-gp assay provides an important early predictor of potential downstream DDI.

In Vitro P-Glycoprotein (MDR-1) Activity Assay, Coupled with Cyp Induction and Hepatotoxicity Assessments, Is an Early Predictor of Drug-Drug Interaction Liabilities

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Rod-shaped, adult canine cardiomyocytes (ACCs) are widely used as an in vitro test system in the fields of cardiosafety (e.g., to test if compounds cause arrhythmias) and drug discovery (e.g., to test if compounds may have beneficial effects in heart failure). However, current methods for quantifying drug effects on contraction in ACCs are low-throughput, as the cell preparations contain dying cells (round shape) vs. healthy cells (rod shaped), and investigators must manually search through the preparations and identify healthy cells for subsequent analysis. We developed high throughput methods for analysis of contraction function in ACCs using the Kinetic Image Cytometer® (KIC), which acquires digital movies from cells at a high frame rate (e.g., 30 to 1,500 frames per second) and which features pacing electrodes. To quantify contractile function at the level of calcium transients, ACCs were seeded into 96-well plates, loaded with Fluo-4, and incubated with test compounds. The KIC navigated to each well, autofocused, paced at 1 Hz for 60 seconds (to equilibrate the contractile function), and then recorded at 30 fps for 10 seconds (while continuing to pace—totaling ~ 80 sec per well). Video analysis algorithms were developed to recognize the healthy ACCs and simultaneously quantify calcium transients and contractile movements on a cell-by-cell basis. Analogously, action potentials and contractile movements were quantified from the ACCs using the fluorescent voltage indicator BeatST1. High throughput methods were also developed to quantify contractile motion of the ACCs for cells in which thioflavin-T was added with whole blood microsomal fractions (featuring conjugated fluorophores) and to quantify both contractile motion (edge detection) and sarcomere shortening for ACCs illuminated in bright field (label-free analysis of contraction). These KIC methods eliminated manual intervention during plate scanning and demonstrated strong positive effects of isoproterenol on calcium transients and contractile movements of the ACCs. The high throughput KIC techniques thus increase the number of ACCs analyzed for greater statistical power, and the speed of the experimental workflow using this cell type for cardiosafety and drug discovery.

High-Throughput Analysis of Contractile Function in Adult Canine Cardiomyocytes via Kinetic Image Cytometry

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Cardiovascular toxicity is a prominent reason for late-stage failures in drug development. Current in vitro cardiovascular safety testing primarily focuses on ion channelopathy and cardiomyocyte (CM) contractility measurements. In recent years, human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have been widely adopted as a physiologically relevant cell model for cardiac safety/toxicity assessment due to displaying a repertoire of ionic currents and channels. However, the contractile apparatus of hiPSC-CM as well as the calcium handling mechanisms are still reminiscent of fetal phenotypes and therefore limit their ability to be utilized for assessment of compounds which modulate the force of contraction (Roberson C. et al, 2013). In order to overcome this limitation we applied long-term electrical pacing using a high throughput pacing apparatus, which can electrically stimulate up to 6 96-well plates simultaneously, to enhance the functionality of hiPSC-CM. Human iPSC-CMs were subjected to continuous and progressive increase in applied electrical pacing for 15 days. The contraction of hiPSC-CMs were recorded using impedance measurements and calcium dynamics was evaluated using Ca2+ transient assays which measures the intensity change of fluorescent Ca2+ dye. Our data clearly demonstrate that 1) hiPSC-CMs possess an inherent negative impedance amplitude-frequency relationship which is reversed after electrical pacing; 2) contraction of paced cardiomyocytes as measured by impedance amplitude increased by positive inotropic compounds and reduced by negative inotrope; non-paced cells either didn’t show any apparent responses to positive inotropes or only showed negative response; 3) calcium handling machinery was improved in paced cells as reflected by significant increase in amplitude of Ca2+ transient peak after caffeine treatment, while non-paced cells showed no response. In addition, enhanced Ca2+ transient response to isoproterenol was observed in paced hiPSC-CMs demonstrating 100% increase in transient amplitude of Ca2+ transient peak compared to 10% increase in non-paced cells. In summary, our data suggests that contractile maturation of hiPSC-CMs can be improved after employment of chronic electrical pacing, resulting in enhanced cell response.

Assessment of Cardiac Contractile Force Modulator Using Functionally Mature Human iPSC-Derived Cardiomyocytes

X. Zhang1, M. Yu2, A. Okonomopoulou3, C. Willits4, K. Green2, and Y. A. Abassi1,1ACEA Biosciences Inc., San Diego, CA; and 2Myokardia, San Francisco, CA; Sponsor: C. Jin

1668 In Vitro P-Glycoprotein (MDR-1) Activity Assay, Coupled with Cyp Induction and Hepatotoxicity Assessments, Is an Early Predictor of Drug-Drug Interaction Liabilities

1667 Investigation of the Mechanism of a Small Molecule-Associated Coagulopathy

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Although prothrombin time (PT) and activated partial thromboplastin time (aPTT) are routinely measured to assess blood coagulation in preclinical general toxicity studies, there are few reports of small molecule off-target, which results in clinically significant prolongation of these screening tests. However, after 7-day repeat dosing of compound X in the Cynomolgus monkey, dose- and time-dependent increases in PT and aPTT were identified, which correlated with clinical signs of melena and hematomas; and macroscopic/microscopic evidence of hemorrhage in multiple organs were noted at all doses. These findings were not recapitulated in rats at comparable exposures. To determine the mechanism of toxicity, more in depth coagulation studies were performed using aliquots of banked citrate plasma. Coagulation factor assays revealed marked decreases in Vitamin K (Vit) K-dependent factors (FII, FIX, FXI, Protein C) without significant decreases in non-Vit K-dependent factors (FV, FXII), suggesting selective perturbation of Vit K metabolism. Thrombin generation was markedly decreased, consistent with the profound decreases in Vit K-dependent procoagulant factors and clinical signs and histologic findings of hemorrhage. There was no evidence of fibrinogen deficiency or dysfunction. When high-dose group plasma was mixed with control group plasma (1:1), PT corrected to normal, indicating that hemorrhage and protracted clotting times were due to factor deficiencies and not inhibition. To determine whether similar coagulation abnormalities could be generated in vitro, normal monkey or human plasma was spiked with compound X before coagulation testing. No compound-induced in vitro prolongation of clotting times occurred, demonstrating that compound X does not directly interact with clotting factors. Our results suggest that compound X may impair the activity of recycling enzymes associated with hepatic Vit K metabolism and/or interfere with Vit K intestinal absorption.

1669 Assessment of Cardiac Contractile Force Modulator Using Functionally Mature Human iPSC-Derived Cardiomyocytes

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to positive inotropic compounds and improvement of calcium handling prop-
erties, which provides a more enhanced model system for assessment if in-
tropic compounds.

1670 Models and Strategies for Building Diversity and Inclusion in Toxicology
C. Curran, Northern Kentucky University, Highland Heights, KY.

Diversity and inclusion in science are more than a strategic goal for univer-
sities, government labs, and industry. Recent findings demonstrate that
diverse scientific teams produce more innovative science and more highly
 cited publications. However, many institutions struggle with implementing
effective programs to increase representation by women, underrepresented
minorities, and those from countries with fewer educational and scientific
resources. This session will bring together leading experts from the Society of
Toxicology, the National Institutes of Health, and the University of Maryland,
Baltimore County’s Meyerhoff Scholars Program to share successful models
and strategies for recruitment and retention of STEM trainees and career de-
velopment toward independent research careers. The topics to be discussed
include (1) an overview of the SOT Undergraduate Diversity Program’s 30
years of experience in recruiting new toxicology trainees and the Committee
on Diversity Initiatives’ efforts to expand global opportunities in toxicology
training, (2) the NIH perspective on supporting career success in biomedical
research where the number of underrepresented minority trainees is increas-
ing while success at the early career stage is lagging, (3) the successful imple-
mentation of the Meyerhoff Scholars Program, which encourages positive
peer pressure among highly capable underrepresented minorities and struc-
tured mentoring toward advanced degrees in STEM fields, and (4) guidance on
successfully navigating a career transition from academia to industry. The
session will conclude with questions from the audience and a general discus-
sion of inclusion and diversity in toxicology training and mentoring.

1671 “Not Your Father’s ED”: Expanding the Definition and Understanding of Endothelial
Dysfunction (ED) Due to Inhaled Toxicants
D. Conklin, University of Louisville, Louisville, KY.

Endothelial Dysfunction (ED) has referred traditionally to any decrement in
endothelium-dependent vascular function. This typically involves nitric oxide
(NO) production/signaling, as NO is one of the most ubiquitous messengers.
This selective definition remains important today, as loss of NO production/
bioavailability can contribute to a number of pathological sequelae, in-
cluding hypertension, erectile dysfunction, and thrombogenesis/stroke. Our
understanding of endothelium biology and ED now incorporates other dys-
functional changes, including angiogenesis, vascular permeability, release of
matrix metalloproteinases (MMPs), expression of adhesion proteins, and loss of
endothelial repair, to name a few, into an ever more inclusive definition of
ED. New technologies and tools applied in the assessment of said func-
tions have spurred innovative measures of myriad ED-related outcomes and
a better appreciation of the functional capacity of endothelium. The session
will span a continuum of complementary methodological approaches, from
flow-mediated dilatation in humans to angiogenesis assays in vitro and in vivo.
As an overall theme for exploring emergent measures of ED, the symposium
will focus on ED induced by inhaled toxins, which provides a real-world, con-
temporary problem and an unresolved mystery by which inhaled air toxics
air pollution, nanoparticles, e-cigarette aerosols) exert extrapulmonary influ-
ence over the systemic endothelium.

1672 Diverse Methodological Approaches to Investigate the Endothelial Impacts of
Toxicants and Systemic Inflammation
M. J. Campen, University of New Mexico, Albuquerque, NM.

The vascular endothelium exists as a barrier between systemically-absorbed
toxicants and target organs, and is therefore a crucial modulator of toxicity. Our
laboratory has developed a battery of in vitro and ex vivo assays that rely on
in vivo exposures to ensure realistic delivery and inclusion of off-target out-
comes such as generation of secondary inflammatory factors. These assays
additionally permit translational correlates and interspecies comparisons. Our
assays address four crucial functions of endothelial cells: regulation of vascu-
lar tone, inflammation, barrier integrity, and wound healing/angiogenesis.
These assays rely on serum, plasma or whole blood from exposed research
animals or clinical subjects. Incubation of endothelial cells or isolated vessels
with serum allows for interaction of all solute factors with the endothelial
surface receptors. Applying such models, we have established that inhaled
toxicants (nanotubes, ozone, diesel exhaust) drive systemic inflammatory sig-
nals carried by the circulation, and this outcome is consistent in humans and
laboratory models. Application of serum or plasma to endothelial cells and
vessels to assay vascular health impacts has a clear role in discovery-oriented
environmental and occupational health research, but may also have value in
clinical diagnostics, prognostics, as well as safety assessments for novel phar-
macaceuticals and supplements.

1673 Flavored Additives in Tobacco Products Induce Endothelial Cell Dysfunction
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Conklin

Use of electronic cigarettes (e-cigarettes) has increased exponentially over
the last decade, and flavored tobacco products greatly increase appeal to
youth. Flavored additives in tobacco products are deemed safe for inges-
tion, but the cardiovascular effects of inhaled flavors are unknown. We devel-
oned a screen of endothelial cell (EC) toxicity to examine effects of flavorings.
Freshly isolated EC from users of menthol-flavored tobacco cigarettes had
impaired A23187-stimulated nitric oxide production compared with EC of
non-smokers. Treatment of EC of non-smokers with either menthol or eu-
genol decreased A23187-stimulated nitric oxide production. Additionally,
human aortic EC were incubated with vanillin, menthol, cinnamaldehyde, eu-
genol, dimethylpyrazine, diacetyl, isoamyl acetate, eucalyptol, and acetylpyr-
azine (up to 100 mM) for 90 min, and cell death, reactive oxygen species pro-
duction, expression of the proinflammatory IL-6, and nitric oxide production
were measured. Some flavors (vanillin, menthol, cinnamaldehyde, eugenol,
aceetylpyridine) induced inflammation and impaired A23187-stimulated ni-
tric oxide production consistent with endothelial dysfunction; while some in-
duced cell death and reactive oxygen species at high concentrations. Heating of
the flavors to temperatures as in e-cigarettes did not alter EC toxicity. Our
data suggest that exposure of EC to flavoring compounds used in tobacco
products may induce adverse EC effects relevant to cardiovascular toxicity.

1674 Air Pollution and Endothelial Progenitor Cells (EPCs): Measuring Loss of Endothelium
Repair Function
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Louisville, KY.

Exposure to fine particulate matter (PM2.5) air pollution is associated with
the depletion of circulating endothelial progenitor cells (EPCs) as well as
vascular injury and dysfunction. It remains unclear if PM2.5 exposure affects
EPC function. Thus, we studied the effects of (and the mechanisms whereby)
PM2.5 on EPC-mediated recovery of tissue perfusion after hind limb ischemia
(HLI) – an in vivo assay of angiogenesis. In comparison with EPCs isolated
from mice breathing filtered air, EPCs from mice exposed for 6h per day for 9
consecutive days to concentrated ambient PM2.5 (CAP) had defects in both
proliferative and tube formation functions. However, 9-days of CAP exposure
in mice overexpressing lung extracellular superoxide dismutase (ecSODTg)
did not affect circulating EPC levels, VEGF-stimulated Akt phosphorylation in
the aorta, plasma NOx levels or EPC tube formation as in WT mice. EPCs from
WT mice exposed to CAP failed to augment basal recovery of tissue perfusion
when injected into naive mice subjected to HLI; an effect absent in EPCs from
ecSODTg mice exposed to CAP. Exposure to PM2.5 impairs EPC abundance
and function and prevents EPC-mediated stimulation of vascular recovery after
HLI. This defect is attributed to pulmonary oxidative stress and endothe-
lium dysfunction.

1675 Endothelial Heterogeneity: Diverse Anatomic and Physiologic Determinants of
Toxicological Assessments after Inhalation Exposures
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The endothelium is a single layer of cells that line the entire cardiovascular
system. Because of the considerable heterogeneity in the cardiovascular sys-
tem, endothelial structure and function can be quite different among discrete
sections. Notably, the microcirculation, which is comprised of all the vascula-
ture contained within a given tissue (arterioles, capillaries, venules and lym-
phatics) possesses diverse endothelial heterogeneity. We have developed mi-
crovascular techniques to directly assess the impact of toxicant exposures on
endothelial function. Intravital microscopy is a whole-animal in vivo approach
used to directly observe microvascular function in skeletal muscle, the mesentery and uterus (virgin and pregnant). Microvessels can be dissected from any tissue and studied ex vivo with the isolated, perfused and pressurized arteriole technique. These collective approaches have been used to assess extrapulmonary toxicity after inhalation exposure to diverse toxicants, including engineered nanomaterials, particulate matter and 3D-printer emissions in various stages of development and pregnancy. The diverse impacts of such toxicities will be discussed in terms of regional and segmental endothelial heterogeneity. Physiologically, this alters mechanotransduction and volume blood flow; which in turn compromise the ability to maintain cardiovascular homeostasis. The impact of these microvascular toxicities will be discussed from a bench-to-bedside, cardiovascular health perspective.

1676 Stem Cells and Metals Toxicity: From Tissue Regeneration and Repair to Carcinogenesis

E. Tokar, NIEHS, Research Triangle Park, NC.

Humans are exposed to metals on a daily occurrence. These exposures can occur during all stages of life, and could result in toxic effects in all organ systems, leading to aberrations in development and other biological processes. Stem cells (SCs) are highly specialized cells that are found in nearly all tissues and organs. Alterations in normal SC functions can adversely affect development and health, and can play key roles in disease etiology. The objective of this session is to highlight the various ways that metals can target and alter SCs during biological processes such as differentiation, tissue repair and regeneration, and carcinogenesis. The first presentation will focus on how chronic arsenic exposure alters muscle SC function to diminished regeneration, and how the effects of this metalloid on the microenvironment appear to play a key role in these alterations. The second presentation will discuss the effects of early-life methyl mercury exposure on muscle development and resultant motor function deficits, including the key roles of several candidate genes that act at the level of muscle SCs and/or regulate myocyte differentiation, mitochondrial biogenesis, and mtDNA transcription. The third talk will describe how subchronic manganese exposure affects adult neurogenesis by reducing the number of neural SCs in the hippocampal dentate gyrus and inhibiting the differentiation of neural stem/progenitor cells to mature neurons. The implications of these effects in Parkinson’s disease will be presented. The fourth presentation discusses how arsenic targets SCs and alters SC signaling pathways during carcinogenesis and the emerging evidence of how the metalloid can “recruit” SCs via altering exosome cargo and the tumor microenvironment. The final presentation focuses on how the stage of life during cadmium exposure can lead to differences in the number of breast SCs and their differentiation. Mechanisms underlying these differences and in breast cancer formation induced by this heavy metal will be presented. This session will be of interest to scientists involved in SCs, metals, development, or cancer research, and will be ideal for those who desire mechanistic understanding of the pathogenic and toxic effects of metals on SCs.

1677 Arsenic-Induced Alterations in the Muscle Extracellular Matrix Drive Stem Cell Dysfunction and Impaired Regeneration


Arsenic (As) contaminates drinking water from natural deposits in the earth. Over 140 million people worldwide are exposed to drinking water that exceeds government As standards. Increasing attention has been paid to declines in functional mobility resulting from chronic As exposure, which poses a significant risk for causing skeletal muscle myopathies and atrophy, impairments that are among the greatest factors contributing to declines in functional mobility and strong predictors of mortality. However, questions of health impacts of life-long environmental exposures remain, including mechanisms by which exposures negatively impact the cellular microenvironment, stem cell (SC) phenotype, and tissue maintenance and healing capacity. Studies of muscle pathology in mice following As exposure via drinking water (10-100 µg/L for 5 weeks) demonstrate As vitiates muscle SC (MuSC) function, resulting in a severely diminished regeneration and force recovery after injury. The microenvironment (niche) is critical for supporting muscle maintenance and MuSC regenerative potential. Niche alterations, including matrix disruption, fibrosis, and ectopic lipid droplet accumulation, often precede and/or accompany declines in the muscle maintenance and healing capacity. The influence of the niche on tissue regeneration involves tightly regulated, bi-directional communication between resident SCs and micro-environmental factors, including extracellular matrix (ECM) components and neighboring cells, including connective tissue fibroblasts (CTFs). We have shown As disrupts the myomatrix by promoting perivascular fibrosis, driving ectopic lipid accumulation and reorganizing ECM integrity. This increased ECM depo-

sition is consistent with impaired myofiber regeneration. Our findings further suggest CTFs isolated from As-exposed muscle exhibit a phenotype with elevated expression ECM remodeling proteins. Moreover, myomatrix alterations following As exposure contribute directly to an impaired muscle healing response after injury. When naive human MuSCs are seeded onto decellularized ECM constructs derived from As-exposed muscle, there is an increased fibrogenic conversion of MuSCs, as compared to MuSCs seeded onto control constructs. These findings suggest As-induced alterations of the myomatrix and matricellular regulators inhibit MuSC function and impair skeletal muscle regeneration.

1678 Muscle Stem Cells and Myogenic Targets of Methylmercury Toxicity

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Methylmercury (MeHg) is an environmental toxin that targets the developing nervous system. Animal and human population studies show that early life MeHg exposure elicits motor and cognitive deficits. Mechanistic studies have therefore focused on neuronal targets. Thus, the possibility that motor function deficit can stem from MeHg alteration of muscle development has been largely overlooked. Our prior screens for developmental MeHg susceptibility genes with Drosophila have identified several genes that constitute core muscle developmental pathways that act at the level of muscle stem cells, namely, the Notch receptor pathway and myoblast fusion genes such as Kirre and Sticks and Stone. We have also observed overt MeHg muscle phenotypes including perturbation of myogenesis in the fly embryo that parallel adverse effects on developing motor neurons. At later developmental stages, MeHg perturbs formation of adult indirect flight muscles during pupal metamorphosis, which also results in a deficient eclosion behavior. We have now extended our investigation using a transgenic strategy of targeting Mrp1 transporter expression in a tissue specific manner to induce MeHg export and cell autonomous protection against MeHg toxicity. Targeted Mrp1 expression in muscle lineages gives a greater overall developmental tolerance to MeHg than Mrp1 expression in neural lineages. Additional functional assays in transgenic flies have identified a downstream target gene of the Notch receptor, the bhlh transcription factor Enhancer of Split mDelta that mediates MeHg toxicity in muscle development. Further studies on mammalian myotube formation using the C2C12 mouse myoblast model find a concentration-dependent inhibition of myoblast differentiation and fusion events. MeHg specifically and persistently affects expression of myogenin, a transcription factor involved in myocyte differentiation. Moreover, factors involved in mitochondrial biogenesis and mtDNA transcription and translation show a decreased expression with MeHg, implicating mitochondria in mediating MeHg-induced change in myogenic differentiation. Ongoing studies will elaborate myogenic phenotypes following prenatal exposure in mice. By characterizing a myopathic component of MeHg neurotoxicity we are expanding the current understanding of how motor deficits are likely to arise from early life MeHg exposure.

1679 Toxicity of Mn Exposure on Neural Progenitors and Adult Neurogenesis

W. Zheng, Purdue University School of Health Sciences, West Lafayette, IN.

Adult neurogenesis taking place in brain hippocampal dentate gyrus (HDG) functions to supply newborn neurons for normal brain functionality. Our published data establish that subchronic Mn exposure can disrupt adult neurogenesis in subventricular zone (SVZ). This presentation further provides the evidence that Mn exposure disturbs adult neurogenesis in HDG. To trace newly generated neural progenitor cells, adult rats were given a single injection of BrdU at the end of 4-wk Mn exposure. Immunostaining data revealed that the majority of BrdU signals were co-localized with S0X2, a selective marker for newly generated neural stem/progenitor cells. Mn exposure caused a 30% reduction of these cells in HDG as compared to controls. To track the fate of BrdU-labeled cells in the HDG, another set of adult rats received BrdU injections for 3 consecutive days followed by 2- or 4-wk Mn exposure. A significant time-dependent migration of newborn cells from the S0X2 toward the granule cell layer was observed in both control and Mn-exposed HDG. Triple-stained neuroblasts and mature neurons further revealed that Mn exposure significantly inhibited the differentiation of immature neuroblasts into mature neurons in the HDG. Based on these observations, the implication of an impaired neuronal repair mechanism in Mn-induced Parkinsonian disorder and possible therapeutic target will be discussed.
Emerging evidence suggests that stem cells (SCs) are a key target cell population for arsenic carcinogenesis. A cancer SC (CSC) overabundance is seen in rodent tumors induced by transplacental and whole-life exposures to arsenic and in human adult (differentiated) cell lines malignantly transformed by the metalloid. Furthermore, human SC lines rapidly acquire a CSC phenotypic barrier in the presence of high-level arsenic exposure. This CSC-like phenotype is characterized by increased self-renewal, drug resistance, and clonal expansion. The presentation will start with a discussion of the various mechanisms involved in arsenic-induced CSC formation and overabundance, including the dysregulation of SC population dynamics, the alteration of tumor suppressor genes (TP53), and key signaling pathways (i.e., Wnt, Sonic Hedgehog, Notch) involved in SC CSC regulation, and the epigenetic regulation of the KRAS oncogene. Next, the presentation will describe how arsenic impacts the microenvironment through SC “recruitment” by altering extracellular and cell membrane cargo, including oncogenes (KRAS, VEGFA, MYB, EGFR), inflammation-related (COX2, IL1B, IL6, TGFβ1, TNFA), apoptosis-related (CASP7, CASP9, BCL2) genes, and cancer-associated microRNAs. Some of the effects described above are shared with other carcinogenic metals, while other effects appear to have opposing effects on SCs. The presentation will end with a brief comparison of the SC-related effects between arsenic and other carcinogenic metals, including cadmium, nickel, and chromium.

Systems Toxicology Approach to the Science of Safety Evaluation
M. Davis, Bristol-Myers Squibb, Princeton, NJ.

Systems toxicology is a transformational subdiscipline within toxicology that applies approaches from systems biology to toxicity-related questions. The session will bring together several advances within systems toxicology that are focused on diverse applications and opportunities in drug safety. Each presenter will share examples of successes and challenges they have experienced with applying “omic methods, definitive approaches (e.g., CRISPR, knockouts/ins), and network analysis to toxicology evaluations. The first talk will provide an overview of the challenges inherent in extrapolating safety signals across species to understand human risk. Immune responses provide a natural model to facilitate our understanding of complex and interactive events, and the second talk will address immunotoxicity in the context of systems approaches that can be applied to understanding the complexity of immune system interactions. The third presentation will focus on the promise and challenges of microphysiological platforms in systems toxicology. The final talk will provide mechanistic insights into species-specific metabolism, with emphasis on how systems approaches can facilitate the selection of biomarkers consistent with rat and human biology. Key insights about how computational models can serve as platforms for contextualizing experimental data and making functional predictions will be shared. The collective content of the session will highlight how we might use sophisticated, integrated systems and modeling to inform safety decisions in drug discovery.

Application of a Microphysiology (MPS) Platform for Systems Toxicology
D. Taylor, University of Pittsburgh, Pittsburgh, PA. Sponsor: M. Davis

This talk will focus on a platform developed for constructing human organs on chips including the evolution of human, 3D, microfluidic, MPS devices to model toxicology for drug development and to model progression of diseases for drug discovery using quantitative systems pharmacology (QSP). To mimic in vivo complexity and functionality of the liver for studying drug efficacy, metabolism and toxicity a 4-cell type liver MPS platform has been developed. In addition, IPCS-derived liver cells from patients enable capture of heterogeneity of genetic and disease backgrounds. The liver MPS can be used as a stand-alone liver or coupled to other organ MPS such as the intestine and pancreas through the vascular channel. It is also possible to quantify immune cell infiltration in the liver MPS along with its applications in toxicology and non-alcoholic fatty liver disease. The critical need to implement an MPS database to manage, analyze and model the data will also be discussed.
The comparative analysis of metabolic networks can provide mechanistic understanding of species-specific differences of metabolism and associated biomarkers and drug targets for various applications. The laboratory rat has been used as a surrogate to study human biology for more than a century. We have generated the first genome-scale reconstruction of Rattus norvegicus metabolism, iRno, and a significantly improved reconstruction of human metabolism, iHsa. Comparative analyses with these models captured metabolic features that distinguish rats from humans. After reconciling network differences between iRno and iHsa, we integrate toxicogenomics data from rat and human hepatocytes to generate biomarker predictions in response to 76 drugs. We validate comparative predictions for xanthine derivatives with new experimental data and literature-based evidence delineating metabolite biomarkers unique to humans. Our results provide mechanistic insights into species-specific metabolism and facilitate the selection of biomarkers consistent with rat and human biology. These models can serve as powerful computational platforms for contextualizing experimental data and making functional predictions for clinical and basic science applications.

Brain health is essential for human well-being across all life stages. Brain development and function are impacted by both genetic and environmental factors. Environmental factors, including exposure to environmental contaminants, are implicated in the etiology of a number of developmental, psychiatric, and neurodegenerative disorders. The zebrafish is a powerful model for assessing the impact of toxicants on brain development and function. Zebrafish embryos are externally fertilized, which enables direct exposure of the developing embryo, obviating the requirement for maternal exposures. In addition, developing embryos are transparent, which allows for in vivo imaging of the developing brain. Overall, development occurs rapidly, including formation of the nascent nervous system by three days of life. Furthermore, a suite of behavioral assays have been developed as functional readouts of toxicant effects on nervous system development and function, and these have been routinely adapted to medium-to-high-throughput screens for hazard identification and chemical prioritization. In this session, researchers will describe how they have leveraged the zebrafish model to investigate different mechanisms of action by which toxicant exposure alters brain development and function. The first presentation will reveal the essentiality of the aryl hydrocarbon receptor (AhR) in blood-brain barrier (BBB) formation and how AhR agonists perturb BBB development. The second talk will introduce the microbiota-gut-brain axis and how developmental exposure to exogenous estradiol compromises neurobehavioral development in a microbiota-dependent manner. The third talk will illuminate the role of GABAergic perturbations by environmental chemicals using a gene editing approach. The fourth presentation will show a mechanistic link between domoic acid exposure, myelination defects, and impaired startle response. The fifth talk will highlight new analytical chemistry approaches that can be used to elucidate mechanisms by which xenobiotics disrupt cholinergic, serotonergic, dopaminergic/adrenergic, histaminergic, and glutamnergic/GABAergic neurotransmitter systems. An excellent 2018 zebrafish session surveyed the multiple uses for larval and adult zebrafish including screening environmental chemicals for developmental toxicity, identifying epilepsy drugs, examining chemical-microbiota interactions when estimating chemical hazard. This abstract does not necessarily reflect US EPA policy.

The brain demands a continuous supply of oxygen and nutrients, while at the same time protecting from blood-borne substances and toxicants. Specialized brain endothelial cells along with neurons, astrocytes, pericytes, and the basement membrane form the blood brain barrier (BBB); a combination of physical and chemical barriers that regulate the entry of blood-borne substances into the brain. Previous research demonstrated that the aryl hydrocarbon receptor (AhR) is necessary for mammalian vascular development and is activated in the zebrafish vasculature following exposure to AhR agonists. To test whether global AhR activation disrupts neurovascular development, we exposed zebrafish to the prototypical AhR agonist, 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). TCDD exposure resulted in cerebral hemorrhaging, neurovascular malformations, and loss of pericytes. To determine whether AhR activation also disrupted BBB development, we used transgenic zebrafish to examine glucose transporter 1 (glut1) expression, a marker of differentiated brain endothelial cells that have acquired barrier properties. In addition, we examined the expression of breast cancer resistance protein (bcrp) and p-glycoprotein (pgp), two important efflux transporters expressed in brain endothelial cells. RT-qPCR results demonstrated glut1 as well as bcrp were significantly reduced following TCDD exposure. However, pgp expression was unchanged. We are using transgenic fish to determine if endothelial-specific activation of AhR recapitulates the observed phenotypes. Together, our results indicate that global AhR activation impairs both neurovascular development and BBB formation. Given that ability to visualize BBB development and function in real time and in vivo in zebrafish, the model is poised to significantly contribute to our understanding of the developmental genetics underlying barrierogenesis, facilitate the development of strategies for manipulating barrier properties to treat disease and effectively deliver drugs to the central nervous system, and provide insight into how toxicant exposures contribute to BBB dysfunction. Supported by NIHES RO1ES023848.

Estrogenic chemicals are widespread environmental contaminants. During early vertebrate development, estrogen receptor signaling is critical for nervous system function. Because host-associated microbiota influence neurodevelopment and can metabolize xenobiotics, we hypothesized that microbiota can biotransform exogenous estradiol (E2) and modify the E2 effect on neurobehavioral development. To test this hypothesis, colonized zebrafish were continuously exposed to five non-teratogenic E2 concentrations (0.34–3.5 μM) from 1 to 10 days post–fertilization (dpf). At 10 dpf, 165 RNA gene sequencing was used to evaluate changes in microbial community composition and predicted metagenomic function. Locomotor activity was assessed in colonized and axenic (microbe-free) zebrafish exposed to 0.4 or 1.2 μM E2 using a standard light/dark behavioral assay and zebrafish tissue was collected to conduct targeted and non-targeted mass spectrometry. E2 exposure did not alter microbial composition or function in colonized larvae. However, light-phase locomotor hypoactivity was observed in E2-exposed colonized larvae as opposed to normal behavior in axenic larvae exposed to E2. Both colonized and axenic larvae exposed to E2 exhibited hypoactivity in the dark phase. Measured parent E2 concentrations and abundance of predicted direct E2 metabolites (E2 sulfate and E2 glucuronide) were significantly higher in axenic relative to colonized zebrafish, while abundance of putative secondary metabolites (estrone sulfate and estrone glucuronide) was similar in axenic and colonized zebrafish. These data suggest a correlation between chemical-induced neurobehavioral effects and microbiota-based metabolism of exogenous chemicals. More broadly, this work supports the inclusion of chemical-microbiota interactions when estimating chemical hazard. This abstract does not necessarily reflect US EPA policy.
There is growing appreciation for the potential of chemical exposures to trigger seizures, which can result in acquired epilepsy. Identifying platforms to screen chemicals for neurotoxic potential and elucidating the mechanisms by which chemicals disrupt the balance of excitatory and inhibitory circuits in the brain to trigger seizures, remain critical data gaps. There are also significant questions regarding mechanism(s) by which chemicals interact with genetic susceptibilities to influence seizure thresholds. Our lab is leveraging larval zebrafish as a powerful model for addressing these questions. Larval zebrafish express the full genetic complement of ionotropic and metabotropic glutamate receptors, GABA(A/B) receptors, and associated regulatory proteins that are expressed in mammalian systems. However, zebrafish offer significant advantages over rodent models, in terms of adaptability to higher throughput technologies and genetic tractability. This presentation will describe the use of larval zebrafish to study the molecular mechanisms by which chemicals trigger seizures, and to screen chemicals for seizureogenic and anti-seizure activity. Topics to be covered include: (1) the discovery of unique patterns of electrical spike activity elicited by chemically distinct GABA(A) receptor blockers, (2) the development of a locomotor assay in larval zebrafish as a higher throughput behavioral assay to screen chemicals for seizureogenic and anti-seizure activity.

### Early Developmental Exposure to Low Levels of Domoic Acid, a Harmful Algal Bloom Toxin, Disrupts Myelination, Leading to Behavioral Effects

J. Panlilio, N. Aluru, and M. E. Hahn, MIT/Woods Hole Oceanographic Institution (WHOI), Woods Hole, MA.

Harmful algal blooms (HABs) produce toxins such as domoic acid (DomoA) that accumulate in seafood and affect human health. While regulations prevent the harvest of seafood with concentrations causing acute toxicity, studies in rodents have shown that low-level exposure to DomoA during early development can have long-lasting, adverse effects on neurodevelopment. We used transgenic zebrafish to identify the cellular and molecular mechanisms of developmental toxicity including cell type targets identified by DomoA, perturbations to nervous system structure, and effects on behavior. We exposed zebrafish to DomoA (0.14-0.18 ng/embryo) by caudal vein microinjection. Exposure to DomoA at 2dpf, but not 1 or 4dpf, caused myelination defects ranging from thinning to the absence of myelin sheaths. Time-lapse imaging showed that DomoA exposure at 2dpf perturbs the initial formation of myelin sheaths from 2.5-3dpf. Exposure to DomoA at 2dpf reduced the number of myelinating oligodendrocytes but not oligodendrocyte precursor cells. Whole embryo RNA sequencing showed differentially expressed genes associated with myelin (mbp, mbpa) and axon cytoskeleton (nelfa, nelfb, nefma, nefmb), suggesting disruption of pathways necessary for maintaining axonal and myelin sheath structures. DomoA-exposed larvae had an impaired startle response, a behavior that requires proper myelination in the circuit. These findings provide mechanistic insights into neurotoxicity of DomoA exposure during critical periods of neurodevelopment by identifying the vulnerable period of developmental exposure during oligodendrocyte differentiation and myelin sheath formation, characterizing DomoA-induced structural changes in myelin sheaths, identifying underlying changes in gene expression, and determining the functional consequences for startle behavior. The implications of these results for hazard assessments for low-level DomoA exposures in humans during key periods of pregnancy and early childhood development will be discussed. Supported by NIH P01ES021923, NSF OCE-1314642.

### A Novel Approach to Identify Neurotoxic Modes of Action

J. Legradi. Vrije Universiteit, Amsterdam, Netherlands. Sponsor: J. Plavicki

Exposure to neurotoxic chemicals contributes to the development and progression of neurological disorders and negatively impacts the health of ecosystems. However, neurotoxicity testing is not currently required for new chemicals. In addition, best practices to assess the neurotoxicity of new and existing chemicals are unclear. Given the increasing numbers of chemicals in commerce and the physiological and morphological complexity of the nervous system, it is a major challenge to test all substances for their neurotoxic potential. New advanced neurotoxicity assessment strategies are therefore needed to fulfill these demands. We are developing a novel neurotoxicity assessment approach which uses behavioral phenotypes in zebrafish embryos to identify neurotoxic modes of action. For this we exposed zebrafish embryos to 39 compounds including pyrethroids, carbamates, organophosphates, neonicotinoids, phthalates and brominated and fluorinated flame-retardants. We then used a widely used light-dark transition test to identify distinct behavioral profiles of exposed embryos. To link these profiles to neurotoxic MoA we use three different omics platforms. We quantified metabolic changes in neurotransmitter systems including the dopaminergic/ adrenergic, glutamnergic/GABAergic, serotonergic, histaminergic and cholinergic systems. In addition, we performed nontargeted proteomic analyses. The transcriptomic changes were analyzed using a custom Neurotox multiplex qPCR array containing 42 neurodevelopmental genes. Data analyses revealed that metabolic changes on neurotransmitter systems correlate well with behavioral responses. The proteomic profiles were mainly influenced by the timing of exposure (bioactivation). The transcriptomic profile could not be linked to the other omics methods and the behavioral tests. Additional tests with chlorpyrifos-oxon, indicate that this might be caused by feedback mechanism in the neurotransmitter systems. Overall, this work reveals complex relationships that are required for neurobehavioral development and function and elucidated common and unique mechanisms by which environmental chemicals disrupt vertebrate neurodevelopment.

### Emergent Mechanisms of Cytochrome P450 Gene Regulation: Defining an Improved Roadmap toward 21st-Century Pharmacogenomics

A. Annalora, Oregon State University, Corvallis, OR.

To understand the interplay between nuclear receptor (NR) signaling and the expression of phase I, II, and III drug metabolizing systems, one must appreciate the role that endo-xenobiotic exposures play in organizing both gene expression and alternative gene splicing events. The human transcriptome is shaped by an array of different factors, including genetics, chemical exposures, diet, and metabolic diseases; this complex process is mediated primarily by differential recognition of gene promoters and canonical splice-sites by either the DNA Polymerase II transcriptional complex or the spliceosome; the structural organization of these multi-subunit, gene-processing complexes are subject to modulation by ligand-activated NR proteins that allow highly specific cell type or cellular microenvironments. Systems approaches are quickly expanding our ability to assess the impact of endo-xenobiotic exposures on gene expression and metabolism. The goal of the session is to integrate cutting-edge research focused on various systems approaches to toxicology that are providing novel insights into the global determinants of Cytochrome P450 (CYP) gene regulation as needed to synthesize a more coherent model of NR-mediated regulation of both transcription and gene splicing. The workshop will highlight research aimed at deconvoluting the overlapping contributions that endogenous substrate, xenobiotic, and microbiome-mediated metabolism play in crafting cellular responses to the environment, in pursuit of an improved pharmacogenomic framework for advancing both predictive toxicology and precision-based approaches to medicine. The presentations will also explore how alternative model systems, such as the organ-on-a-chip technology, are providing new opportunities to manipulate CYP gene expression and splicing in a highly personalized manner that promises to usher in a new era of safe and effective gene-directed therapeutics.

### Meta-Transcriptomics Analysis of Alternative Splicing in the Cytochrome P450 Superfamily: Decoding Novel Mechanisms of Gene Regulation and Function, Environmental Toxicity, and Disease

A. J. Annalora, Oregon State University, Corvallis, OR.

Alternative pre-mRNA splicing in the cytochrome P450 (CYP) superfamily dramatically expands the complexity of the CYP transcriptome. A meta-transcriptomics analysis of alternative CYP splicing identified nearly 1,000 splice variants among 57 human CYP genes linked to tissue-specific, genetic, chemical exposure and disease. The transcriptome expansion that underlies this phenomenon is mediated by a complex array of cell-specific splicing factors, oxidative stress, heavy metal toxicity and xenobiotic exposures that can alter: 1) the quantity and quality of pre-mRNA; 2) the assembly and stability of the spliceosome; and 3) the activation of nuclear receptor (NR) genes that modulate both gene expression and splicing events based on cues from the environment, metabolome and microbiome (Toxicology. 2012. 296:1–12). CYP proteome expansion arising from this diversity may
1695 Gut Microbiome: A Novel Frontier for Xenobiotic Metabolism in the Host Liver

J. Cui. University of Washington, Seattle, WA.

Accumulating data in the literature have demonstrated the critical roles of intestinal bacteria on the host intermediary metabolism including obesity and type II diabetes. Relatively less is known regarding the impact of intestinal bacteria on the hepatic drug-processing gene expression and xenobiotic metabolism. RNA-sequencing demonstrated that in livers of germ-free mice, there was a marked decrease in the hepatic expression and enzyme activity of cytochrome P450 (CYP) 3A, which is a major class of phase-I enzyme involved in the biotransformation of over two-thirds of the drugs prescribed in the market; in contrast, there was a marked increase in the hepatic expression and enzyme activity of CYP4A, which is important for lipid metabolism. Chromatin immunoprecipitation and qPCR results suggested that this was likely due to attenuated pregnane X receptor signaling but enhanced peroxisome proliferator-activated receptor signaling. During liver development, the presence of intestinal microbiota markedly impacted the normal ontogeny of many hepatic drug-processing genes in a gender-specific manner. Introducing exogenous bacteria by probiotics or conventionalization also influenced the hepatic drug-processing capacities. Finally, the environmental chemical polycyclic aromatic hydrocarbons (PAHs) mediated alterations in many hepatic drug-processing genes depended on the presence of a normal configuration of the gut microbiome, and the germ-free condition altered the amount of certain phenolic metabolites of PAHs in vivo. In conclusion, gut microbiome critically impacts the expression of drug-processing genes and the metabolism xenobiotics of the host liver.

1696 Intestinal Epithelial Cell Receptors as Modulators of Host-Microbiota Communication

A. Patterson. Pennsylvania State University, University Park, PA.

A complex network of host receptors and microbiota within the gastrointestinal tract work in concert to process and absorb dietary nutrients, detoxify xenobiotics, and establish a homeostatic system that regulates metabolism and inflammation. Emerging evidence suggests ligand-activated transcription factors of the nuclear receptor superfAMILY and the basic helix-loop-helix/per-art-sim (PAS) family not only receive and process chemical signals derived from microbial-dependent metabolic activity, but also transmit these signals to distant organs, including the liver. For example, small intestine signaling of the farnesoid X receptor (FXR), an essential regulator of bile acid, lipid, and glucose metabolism, is modulated through gut microbiota-dependent metabolism of bile acid metabolites produced in the liver. Additionally, studies of the aryl hydrocarbon receptor (AhR), a xenobiotic sensor, have revealed microbial metabolites derived from dietary nutrients including tryptophan as critical regulators of both intestinal and hepatic inflammation. Dissection of the host-microbiota interaction has been facilitated by use of transgenic mouse models, host and microbiome sequencing, and mass spectrometry- and NMR-based metabolomics. Identification and characterization of microbial metabolites and their relationship with host receptors will provide new avenues for studying host-microbiota communication networks and identifying new therapeutics to modulate this interaction in human disease.
cochemical properties. If the uncertainty and domain of applicability can be characterized and quantified, then these methods would allow for a timely, risk-based prioritization strategy characterizing dose relationships between in vitro bioactivities and predicted human exposure. Presenters will consider the state-of-the-science between traditional and higher-throughput methods, and the associations between them, such as extrapolation techniques, model confidence, acceptable uncertainty, and context applicability. Understanding the state-of-the-science in in silico toxicokinetics for government and industry applications will aid the inclusion of such techniques when limited data are available.

**W 1700 Implementation and Evaluation of State-of-the-Art In Silico Models for In Vitro and In Vivo Endpoint Predictions**

D. Mucks. RISE (Research Institutes of Sweden), Södertälje, Sweden. Sponsor: J. Wambaugh

Model evaluation and applicability domain determination allow for a timely, risk-based prioritization. Both of these concepts are an important - and often overlooked - part of the quantitative structure/activity/property relationship (QSAR/QSPR) model development process. However, evaluation and domain determination can be directly addressed when using state of the art Machine Learning (ML) and Deep Learning (DL) approaches. Conformal Prediction provides a framework for existing ML and DL methods to address this issue by using a mathematical approach to better estimate prediction reliability through linking the applicability domain to the QSAR/QSPR model in use and not just basing it on training data alone. Recently developed novel DL approaches (i.e. Google TensorFlow, Theano) give promising initial results on a variety of datasets and also provide the opportunity for utilization of transfer learning approaches, meaning simultaneous usage of multiple datasets and endpoints in the model training process. Implementation of such approaches has become easier over the past decade with the popularity and rapid development of freely available high-level programming languages (i.e. Python, R) and workflow engines (i.e. KNIME, Rapidminer). Careful integration of these in silico models into a combined framework with traditional approaches (i.e. in vivo) and kinetic models can give a good overall estimate of uncertainty and reliability for the in vitro and in vivo predictions, allowing uncertainty to be directly propagated into the risk-assessment of novel and data-poor compounds.

**W 1701 Applying In Silico-In Vitro Extrapolation (IS-IVIVE) Techniques to Predict Exposure and Guide Risk Assessment**

M. Lawless. Simulations Plus, Lancaster, CA.

The ability to accurately predict absorption/systemic pharmacokinetics (PK) directly from 2D structure can be very helpful in assessing the risk associated with a drug or chemical. We have developed an in silico-in vitro-in vivo extrapolation (IS-IVIVE) workflow that scientists can efficiently apply to predict compound exposure in animals or humans. This requires the integration of QSAR models to estimate necessary input parameters e.g., fraction unbound to plasma proteins, fraction unbound in tissues, and CYP metabolism, that inform mechanistic absorption/PBPK models. Cytochrome P450 metabolism is a key component of this methodology. We use a series of QSAR models to predict sites of metabolism, metabolites, and kinetic parameters (Km, Vmax, and Clint) for five major human CYP isoforms. This allows one to simulate linear/nonlinear metabolism of the parent compound and track metabolites. We have also recently developed a series of clearance classification models based on the Extended Clearance Classification System (ECCS) which provide additional information that can be useful in identifying which clearance pathways (metabolism, hepatic uptake, or renal) is likely responsible for driving a compound’s elimination. This IS-IVIVE method has been applied to investigate toxicokinetic profiles of various environmental compounds and pharmaceuticals to assist with risk assessment activities, e.g., drug-drug interactions.

**W 1702 Quantitative Property-Property Relationship for Screening-Level Prediction of Intrinsic Metabolic Clearance**

C. Kirman. Summit Toxicology, Bozeman, MT.

A quantitative property-property relationship model was developed to predict intrinsic metabolic clearance for 403 chemicals across a broad range of chemical properties and validated using a published set of metabolic clearance measurements. Measurements were predicted using octanol-water and water-air partition coefficients that are readily available or can be quickly estimated for most chemicals. These two chemical properties were determined to be statistically significant predictors of metabolic clearance, with > 90% of predictions being within a factor of 10 of their measured value, and > 60% being within a factor of 3. The resulting metabolic clearance estimates, with appropriate confidence bounds, can be readily incorporated into a generic physiologically based pharmacokinetic model for a wide range of chemicals, which can be used to estimate steady-state blood concentrations resulting from environmental exposures. The level of precision associated with this approach (i.e., within an order of magnitude) allows a screening level estimation of this important metabolic parameter for a wide range of chemicals.

**W 1703 Designing QSARs for Metabolic Clearance and Plasma Protein Binding in Diverse Chemical Space Using Pharmaceutical Data**

B. Ingle. ICF International, Research Triangle Park, NC.

High-throughput toxicokinetic models can be a useful tool in screening for chemical risk, yet limited experimental data is available for two important parameters: fraction of a chemical unbound to plasma proteins and hepato-cellular metabolic clearance rate. Open source quantitative structure-activity relationships (QSAR) for fraction unbound and metabolic clearance were developed using several machine learning algorithms and curated in vitro data (a library of mostly pharmaceutical compounds with >1000 plasma binding measurements, >3000 metabolism rates). Models were evaluated using both pharmaceutical and environmentally relevant chemicals. Although models were trained largely on pharmaceuticals, most of the environmentally relevant chemicals evaluated within this work were within the applicability domains. Therefore, these models offer reliable in silico predictions for both pharmaceuticals and chemical sets representative of the types of pollutants and products regulated under the Frank R. Launenberg Chemical Safety for the 21st Century Act. The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the US EPA.

**W 1704 Using Chemical Structure Information to Develop Predictive Models for In Vitro Toxicokinetic Parameters to Inform High-Throughput Risk Assessment**

P. Pradeep. US EPA/ORISE, Research Triangle Park, NC.

Toxicokinetic parameters for metabolic clearance and fraction of the chemical unbound to plasma proteins are critical for relating exposure and internal dose when building in vitro-based risk assessment models. However, conducting in vivo toxicokinetic studies to characterize these parameters has time and cost limitations. Data gap filling techniques such as read-across and quantitative structure-activity relationships (QSARs) are commonly used to predict hazard in the absence of empirical data. This talk presents: (1) an evaluation of the suitability and limitations of chemical structure information to predict TK parameters of interest (fraction of chemical unbound) in silico using a dataset of 1487 environmentally relevant chemicals, (2) the utilization of predicted TK parameters within a set of in vitro models to calculate steady-state concentration in plasma to determine an oral-equivalent dose, and its comparison with AroClO exposure estimates for an external set of 235 chemicals, and (3) implications of variability in experimental and predicted TK parameters, and physico-chemical properties on the uncertainty in resultant oral-equivalent dose estimates. Overall, these models could allow for an informed risk assessment and chemical prioritization of data-poor chemicals. The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the US EPA.

**W 1705 Shifting Currents in Predictive Toxicology and Safety Evaluation with In Vitro and Alternative Approaches**

M. Fortin. Jazz Pharmaceuticals, Ewing, NJ.

Transformative approaches, such as organotypic in vitro models and high content screening, are redefining the science of toxicology. However, the path to their integration in toxicity testing programs remains somewhat elusive. Toxicity testing, a necessary element of product development and the foundation of human health risk assessment, has traditionally relied heavily on in vivo animal studies. The advent of in vitro and computational technologies holds promise to drastically change and improve the testing paradigms of the future. Beyond the obvious benefit of reducing animal testing,
embracing high-throughput predictive models can both provide valuable information to aid in molecule design and provide guidance for targeted toxicological testing strategies. This session will focus on innovative methods, such as toxicogenomics, 3D microtissues, and in vitro high content analysis, that are being used to characterize the safety profile of molecules and products, and their application to predictive and mechanistic toxicology testing approaches. Emphasis will be given to methods that are currently being employed to characterize the safety profile of molecules and products and inform decision-making. The targeted audience would be those interested in understanding how these tools are being leveraged in real-world applications, such as integrated approaches to testing and assessment (IATA) strategies to help guide in vitro and in vivo testing programs. To this end, experts from industry, government, academia, and non-for-profit were gathered to discuss the current state-of-the-science. The talks will present how cutting-edge research tools and next-generation alternative models are being integrated in the safety evaluation of environmental chemicals, pharmaceuticals, and plant protection products. Topics covered will include the value of in vitro transcriptomics to predict in vivo apical findings and identify points of departure; the use of a systems approach to predict and mechanistically classify kidney toxicity in vitro; the use of in silico and in vitro models in discovery toxicology; and the utility of 3D tissue models for screening endocrine disruptors. Following the presentations, a Q&A will be held to engage the audience. Attendees will leave with a deeper understanding of the realm of potential applications of next-generation toxicology models. They will also gain insight into the strengths, limitations, and future development opportunities of in vitro and alternative models for predictive toxicology.

**W 1706 Evaluation of In Vivo and In Vitro High-Throughput Transcriptomics for Safety Assessment**

W. Gwinn, NIEHS/NTP, Research Triangle Park, NC.

Initial efforts at high throughput safety assessment focused on assays that evaluated a single pathway, for example estrogen receptor activation. While chemical throughput was considerable in this approach, biological space queried was limited to a single pathway at a time. More recently high throughput transcriptomics has been proposed as an alternative screening approach. While this high throughput transcriptomics covers more biological space, its cost is likely to decrease the number of chemicals that will be queried. In ongoing studies at the NTP, we have evaluated high throughput transcriptomic approaches using short-term in vivo models and in vitro models combined with in vitro-in vivo extrapolation (IVIVE). This initial evaluation has focused on hepatotoxicants that act through a variety of pathways such as genotoxicity, reactive intermediates and receptor mediated mechanisms. Transcriptomic dose response data was analyzed using BMD Express 3.0 and benchmark doses for individual chemicals were estimated based on the median benchmark dose of individual genes in a pathway. The use of transcriptomic screens reveals in points of departures within a factor of 5 compared to points of departures estimated from long term toxicity studies. These data suggest that transcriptomic screening approaches provide reasonable estimates of points of departures for apical endpoints when pharmacokinetics are appropriately considered.

**W 1707 Using a Systems Approach to Predict and Mechanistically Classify Kidney Toxicity In Vitro**

S. Ramm, AstraZeneca, Boston, MA.

The failure to predict toxicity of new chemicals and therapeutics early in the development process before they reach humans remains a critical problem. In this presentation, a systems biology approach using primary human kidney cells, that combines multidimensional datasets and machine learning will be presented. Imaging of 1000 gene and >600 live-cell high-content imaging feature read-outs for a library of well-characterized kidney toxicants (e.g. gentamicin, Cd, cisplatin) were characterized and ranked by their predictive capacity using Random Forest machine learning. The biomarkers we identified not only predicted nephrotoxic compounds with greater accuracy than standard measures of cell death and viability, but could also be validated in 3D kidney models. Network analysis of similarities in toxic phenotypes based on high-content image analysis was also performed, which offered insights into potential mechanisms of toxicity for candidate drugs. Finally, we validated our predictive biomarkers and mechanistic network analyses through their ability to accurately predict kidney toxicity in 4 out of 6 drug candidates that only exhibited toxicity in late stage development. In summary, a new approach to generate panels of biomarkers that can be measured using high-throughput in vitro screening to enhance the accuracy of nephrotoxicity risk assessment.

**W 1708 Integration of In Vitro and In Silico Models for Predictive Toxicology in Discovery Molecule Development**

J. L. LaRocca, Corteva Agriscience, Indianapolis, IN.

The discovery and development of novel molecules is a complex, interdependent process, which requires analysis of thousands of compounds to meet the stringent requirements for animal use. Embracing high throughput predictive models for assessment of potential hazards of molecules early in discovery can help drive in decision-making, with the ultimate goal of generating products of the future with a more favorable human health profile than the past. To this end, we assessed the predictive utility of cytotoxicity endpoints by comparing in vitro IC50 values of 2D and 3D HepG2 cells to reported rat 90-day in vivo NOAEL and LOAELs for 22 pesticides. Using a linear mixed-effects model accounting for a grouping structure of pesticide class (fungicide, insecticide or herbicide), we demonstrated in vitro cytotoxicity are correlated with in vivo endpoints. Given that molecules can have varying absorption and bioavailability profiles, which can impact in vivo vs. in vitro points of departure but are not accounted for in in vitro systems, we integrated PBPK software to aid in placing in vitro cytotoxicity values into a more biologically relevant context for internal exposure. Finally, integration of transcriptomics into early-stage discovery programs can help predict points of departure for risk assessment. In vitro and in vivo points of departure for multiple fungicides were compared to in vivo apical endpoints, which suggest toxicogenomic bioactivity can provide an early indication of in vivo toxicity. These data demonstrate that harnessing in vitro cytotoxicity, in silico PBPK models, and in vitro and in vivo toxicogenomics can predict in vivo points of departure, which can aid in molecule prioritization decisions in the discovery phase.

**W 1709 Screening Estrogenic Endocrine-Disrupting Chemicals with Human MCF-7 3D Microtissues by In Vitro Pathology**

K. Boekelheide, Brown University, Providence, RI.

As outlined in the National Research Council report "Toxicity Testing in the 21st Century: A Vision and a Strategy," there is a need to develop more efficient and physiologically relevant models for evaluation of chemical safety and toxicity. Human MCF-7 breast cancer cell 3-dimensional (3D) microtissue cultures assessed by in vitro pathology are being developed as an improved screening assay for estrogenic endocrine disrupting chemicals (EDCs) compared to traditional 2D models. Growth in agarose hydrogels, these cells self-assemble into microtissues reminiscent of normal in vivo human mammary epithelial tissue with lumen-forming glands, up-regulated cell-type specific differentiation markers, and more complex responses to estrogenic stimulation when compared to the same cells grown in 2D. In vitro pathology assessment of MCF-7 3D microtissues includes a 3D proliferation assay, a differentiated morphology assay, and a transcript assay. The 3D proliferation assay identifies nuclei in 3D microtissue z-stack confocal slices as a measure of proliferation. The differentiated morphology assay detects glandular lumens and then determines their volumes. The transcript assay measures the most significant and highest-fold change transcripts following 3D MCF-7 microtissue exposure to estrogenic pathway agonists (APOD, BMP4, CCNA1, CLDN1, CYP1A1, CYP19A1, ERBB2, ESR1, MYC, PDZK1, TGFb3). This combination of simple and reproducible assays is amenable to high-throughput confocal imaging and molecular analysis, offering a unique opportunity for the safety evaluation of estrogenic EDCs over a broad concentration range.

**W 1710 In Vitro Hepatic Model Systems for Investigative and Predictive Toxicology Applications**

E. LeCluyse, LifeNet Health Institute of Regenerative Medicine, Research Triangle Park, NC.

Prediction of compound-induced hepatotoxicity in humans from in vitro data continues to be a significant challenge for the pharmaceutical and chemical industries. Historically, in vitro 2D hepatic model systems are limited by their inability to maintain phenotypic cellular characteristics over time in culture, especially the stable expression of clearance mechanisms, key bioactivation enzymes, and the cellular interactions during the quenching and resolution of hepatocellular injury. As such, phenotypically stable multicellular systems are required to investigate the key initiating events and the adaptive events, such as inflammation, proliferation, and fibrosis, over prolonged exposure periods. Increasingly sophisticated in vitro humanized test systems and emerging computational models are being introduced to improve our ability to more accurately predict hepatotoxic compounds during development. This workshop segment will present recent advances in tissue culture technol-
ógies and strategies for in vitro-in vivo extrapolation for human risk assessment using quantitative systems pharmacology. Case studies with some of the leading-edge culture devices that represent more physiologically-relevant, organotypic in vitro surrogates of human liver will be discussed. The important role of non-parenchymal cells as targets of toxicity and mediators of hepatotoxic responses will also be highlighted. These emerging culture technologies could have a major impact on global public health. RRs and new computational modeling approaches are drastically improving our ability to accurately predict and understand the hepatotoxic potential of new compounds.

1711 Strategies to Mitigate the Health Impacts of Air Pollutants in Susceptible Populations

H. Tong. US EPA, Research Triangle Park, NC.

The World Health Organization (WHO) has estimated that indoor and outdoor air pollution causes approximately 7 million premature deaths worldwide each year, and 40,000-60,000 premature deaths in the United States alone. Implementation of the regulations of the Clean Air Act has brought forth substantial improvements in air quality and attendant benefits to public health. Yet tens of millions of Americans still live in areas where levels of air pollutants exceed US EPA’s National Ambient Air Quality Standards (NAAQS). Furthermore, some studies have shown that there is no threshold for exposure to particulate air pollution below which exposure is safe, implying that susceptible individuals may be at risk of adverse health effects not only in nonattainment areas but also in communities that are in compliance with NAAQS. Two main intervention strategies to further reduce adverse health impacts of air pollution are reducing personal exposure to air pollution and reducing vulnerability to adverse health effects. Ifair pollution and various intervention approaches have been proposed under each approach, it is uncertain if there is sufficient scientific basis to support their use, or if there are potential health risks associated with their use. The goal of the session is to discuss selected approaches within each of these two strategies. The session will open with a review of US EPA regulations to improve air quality and an overview of interventions aiming to reduce personal exposure to and alleviate adverse health effects of air pollution. This will be followed by five presentations on selected interventions to discuss potential benefits and risks of each strategy. Specifically, interventions focusing on reducing vulnerability to adverse cardiac and pulmonary effects of environmental pollution, such as dietary supplementation strategies and contact with greenery, and interventions focusing on reducing exposure levels to air pollution, such as use of cleaner cookstoves in Guatemala and use of physical barriers and personal behavior changes, will be presented. The session will conclude with a panel discussion with the audience that addresses the following questions: (1) What are the top priorities to reduce the health impacts from air pollution? (2) What are strategies to reduce exposure and vulnerability to adverse health impacts of air pollution at an individual level in susceptible populations? (3) What is a preferred solution to household air pollution: better stoves or cleaner fuels? (4) What are the potential mechanisms for intervention? (5) What measures can be used to mitigate the health impact of air pollution at the community level? (6) What other nontraditional approaches can be used to mitigate the health impact of environmental pollutants? Following this session, attendees will have a better understanding of the research needs and opportunities to deploy such approaches to mitigate damage from air pollutants.

1712 Better Stoves or Cleaner Fuels: What Is the Evidence Base That Is Needed to Decrease the Burden of Household Air Pollution?

J. R. Balmes, University of California Berkeley, and University of California San Francisco, San Francisco, CA. Sponsor: H. Tong

Household air pollution (HAP), arising from the combustion of dirty-burning fuels in an unventilated home for cooking and heating (e.g., wood, charcoal, dung, coal) is estimated by WHO to cause around 4 million premature deaths a year, mainly from cardiopulmonary diseases, making it one of the commonest underlying drivers of morbidity and mortality in low- and middle-income countries (LMIC). Although not included in the WHO estimates, the use of kerosene for lighting contributes additional morbidity and mortality. Acute lower respiratory illness (ALRI) in young children and chronic obstructive pulmonary disease (COPD) in adults remain two of the primary drivers of HAP-related burden of disease, although there is indirect evidence for cardiovascular disease (CVD) as well. The observational epidemiological evidence for the associations between exposure to HAP and the burden of disease has been considered sufficiently strong that intervention trials have been designed and conducted to reduce exposures. Unfortunately, after the initial success of the first randomized controlled trial (RCT) of a cleaner cookstove to prevent child ALRI in Guatemala, results of most of the subsequent RCTs have been disappointing. Given the burden of disease in LMIC, it would be extremely helpful to have better HAP exposure-response data. Stove distribution programs without knowledge about how clean is clean enough may be doomed to failure. Policy makers in resource-limited settings need this information to guide investment strategies to maximize public health impacts.

1713 Vitamin E (Gamma-Tocopherol)-Based Intervention for Environmental Airway Disease

N. Alexis, University of North Carolina at Chapel Hill, Chapel Hill, NC. Sponsor: H. Tong

Epidemiologic evidence has suggested that increased dietary vitamin E intake is associated with reduced incidence of allergic disease and asthma. Alpha tocopherol (aT), an isoform of vitamin E, and commonly available supplement and pharmaceutical product, has shown disappointing results as a therapeutic agent capable of mitigating features of asthma. Gamma tocopherol (γT), however, the predominant isoform of vitamin E found in diets, has been shown to have both anti-inflammatory and antioxidant actions. Our Center has pursued a program of preclinical and early phase clinical trials of γT as a novel therapeutic for treatment of airway inflammation in animal models and human studies. This abstract will review our γT studies in rodent models of pollutant-evoked airway inflammation, as well as our human intervention studies that have examined allergen-induced eosinophilia and mucin responses in asthma. Not only is γT-induced neutrophilia in healthy and asthmatic volunteers. In summary, we have shown that in a rodent model of allergic asthma and air pollutant (ozone and LPS)-induced airway inflammation, γT reduced allergen-induced eosinophilia, mucin responses and LPS-induced neutrophilia, prostaglandin E2, and MUC5AC levels. We have also shown that 1 week of treatment with a γT-enriched mixed tocopherol preparation reduced the neutrophilic response to inhaled LPS challenge in a phase I randomized, double-blinded, placebo-controlled crossover study of healthy adults. In asthmatics, we have observed that a two-week supplementation with γT reduced features of asthma including sputum eosinophils, mucins and the acute LPS-induced neutrophilic response in the airways. In asthma, it is interesting to speculate whether γT can be used as a complementary or steroid-sparing treatment for asthma and in healthy individuals, whether γT is an effective treatment strategy for mitigating the adverse health effect of air pollution exposure.

1714 Dietary Interventional Approach to Ameliorate the Health Effects of Air Pollution

J. M. Samet, US EPA, Research Triangle Park, NC.

Exposure to ambient air pollution is the fourth leading cause of mortality and a major cause of human morbidity around the world. The identification of efficacious interventional approaches to mitigate the adverse health effects of airborne pollutants requires rapid translation to public health impact. While no single physicochemical agent or property of air pollution has been identified as the causative agent, oxidative stress has been proposed as a toxicological mechanism that is common in many disparate components of air pollution. The antioxidant properties of certain vitamins and polyunsaturated fatty acids derived from dietary sources have been shown to confer a broad range of health benefits, suggesting that they might provide protection against the deleterious effects of air pollution. Our studies have shown that in human subjects exposed to fine and ultrafine particulate matter, fish oil supplements containing high levels of omega-3 fatty acids can ameliorate cardiac rhythm changes and improve blood lipid effects, while olive oil pills can protect against aberrations in vascular function. These studies afford mechanistic insight into the toxicology of ambient air pollutants and suggest that dietary interventions are an effective strategy that can protect exposed populations.

1715 Personal Solutions to Air Pollution: What Does the Evidence Recommend?

H. M. Kipen, Rutgers, The State University of New Jersey, Piscataway, NJ.

A substantial proportion of exposure to outdoor-generated (and of course indoor-generated) air pollutants occurs while individuals are indoors because individuals spend most of their time indoors. When concentrations of ambient air pollutants exceed levels associated with increased risk of health problems, air-purifying actions taken to reduce indoor pollution levels and the health effects are sometimes recommended. Beyond behavior change, we have demonstrated that air-filtering techniques are effective at lowering indoor pollutant levels. Such techniques are widely used in the more polluted areas of the
1716 Urban Green Spaces Reduce Stress-Related Allostatic Load and Enhance Resilience to Environmental Insults  
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Exposure to urban green spaces has been linked to reduced cardiovascular morbidity and mortality, diabetes, and depression, as well as improved pregnancy outcomes. Relaxation and chronic stress alleviation, beneficial exposure to natural allergens and microbes, and enhanced physical activity are some of hypothesized pathways to these salutogenic effects. It has been hypothesized that enhancing contacts with natural living environment in urban settings can enhance innate resilience to detrimental effects of environmental stressors including air pollution. US EPA’s epidemiological studies in North Carolina aim to assess the effects of long-term exposure to residential greenness on stress-related physiological dysregulation known as allostatic load. Allostatic load estimates are based on multiple biomarkers of immune, metabolic and neuroendocrine functions. A recently completed pilot study demonstrated a highly significant ($p < 0.0001$) inverse association between residential greenness and allostatic load, as well as reduced odds of having a potentially unhealthy level of individual biomarkers, such as catecholamines, dehydroepiandrosterone, salivary a-amylase, fibrinogen, C-reactive protein, vascular cell adhesion molecule-1, and selected proinflammatory cytokines. These associations persisted after adjusting for traffic air pollution levels and body mass index, suggesting that living in green areas might be a protective pathway to healthy weight loss, stress mitigation and immune system modulation. Other studies demonstrated that exposure to air pollution can affect the same biomarkers in the opposite direction. Thus, this pilot study contributed to a growing body of evidence that contacts with the living nature reduce vulnerability to environmental insults including air pollution. For this ongoing study with an expanded set of immune system biomarkers includes analysis of gut microbiome to assess links between residential greenness, microbiome, and immune function. A prospective component of this study aims to evaluate if reduced allostatic load increases resilience to short-term environmental insults.

1717 Bayesian Evaluation of Physiologically Based Pharmacokinetic (PBPK) Modeling for Perfluorooctanesulfonate (PFOS) to Characterize the Interspecies Uncertainty between Mice, Rats, Monkeys, and Humans: Development and Performance Verification  
W. Chou, and Z. Lin. Kansas State University, Manhattan, KS.
Interspecies differences of toxicokinetic properties of perfluorooctanesulfonate (PFOS) has been shown to result in substantial uncertainty of dosimetry estimation and risk assessment in the extrapolation from animals to humans. The development of physiologically based pharmacokinetic (PBPK) models accounting for the species-specific toxicokinetic parameters of PFOS is desirable. In this study, based on previously published model structure we developed PBPK models for PFOS in mice, rats, and humans after different routes of administration. Available species-specific in vivo toxicokinetic data were used for model calibration and independent datasets were used for model validation. Critical determinants governing tissue distribution were determined by local sensitivity analyses. In addition, Bayesian statistical analysis using Markov chain Monte Carlo (MCMC) were performed to characterize uncertainty and interspecies variability in the chemical-specific parameters. Simulation results suggest that the model predictions were successfully validated across species with estimated residual error of less than 2-fold. Several model parameters, including partition coefficient of liver, urinary and biliary elimination rates of PFOS, and concentrations of apical and basolateral transporters were identified as highly influential factors governing the overall pharmacokinetics. Bayesian MCMC analyses showed that Vmax for apical transporter and urinary/biliary elimination rates varied significantly across species. This study provides an important step toward estimating interspecies uncertainty, improving the extrapolation of dosimetry estimation of PFOS from animals to humans.

1718 Class Comparison Study of Perfluorinated Substances in Sprague-Dawley Rats: Liver Toxicity and Thyroid Hormone Dysregulation  
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Several straight-chain perfluorinated substances (PFAS) were evaluated in a class comparison study to assess the relative toxicity of specific PFAS in relation to their chain length and branch length. Perfluorooctanesulfonate (PFBS), perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), or perfluorodecanoic acid (PFDA) were administered by gavage to male and female Sprague Dawley rats for 28 days at doses designed to maximize the dose response for each individual PFAS. An agonist of PPARα, WY-14,643 (WY), was included for qualitative comparisons. Toxicity was evaluated using complete histopathology, thyroid hormones, clinical chemistry, hematology, andrology, and plasma and liver levels of parent PFAS. Plasma and liver levels were the highest for the longer chain chemicals with the most apparent sex difference displayed by PFHxS, PFOA, and PFDA. Liver/plasma ratios in males were generally at or below 1.0 except for PFOS (greater than 2.7 at all tested doses) and PFDA (greater than 1.6). Apart from PFHxS in females, liver toxicity was observed in all chemicals and was manifested by increased serum liver enzyme activities (except PFHxS) and liver weights, and histologically by hepatocyte cytoplasmic alteration and hepatocyte hypertrophy in all chemicals. Generally, the occurrences of these lesions increased with chain length, and were more frequent in males than females. In animals treated with PFNA and PFDA, the longest chain lengths represented, there were dose-dependent increases in hepatocellular necrosis. Generally, PFAS exposure decreased circulating levels of T3, free T3, and T4 total (up to 90%), with males being more sensitive than females. WY-treated animals exhibited hepatocyte hypertrophy and cytoplasmatic alteration at all dose levels, consistent with the other PFAS, and WY-treated males had decreased circulating free T4. PPARα and CAR activation were evaluated by measuring target liver enzyme expression (Cyp2b1, Cyp2b2, Acox1, and Cyp4a1) and correlating this to various related endpoints. The data presented can be used in determining potency estimates on a variety of endpoints, and future predictions for PFAS toxicity.

1719 An In Vitro Screen of a Panel of Perfluoroalkyl Substances and an In Vivo Assessment of Effects on Placental and Fetal Growth  
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Perfluoroalkyl substances (PFAS) are ubiquitous environmental contaminants and are associated with fetal growth restriction (FGR). Perfluorooctanoic acid (PFOA) is one of the most well-studied PFAS and it is estimated that a 1 ng/mL increase in serum PFOA is associated with an 18.9g decrease in human birth weight. The mechanism through which PFOA affects fetal growth is not known and we hypothesize that the fetal-placental unit is a target. PFOA is one of thousands of PFAS, and determining modes of action against the developing fetus as well as a method for PFAS prioritization are necessary. A high throughput, multiplexed screen was developed using human placental JEG-3 cells for PFAS prioritization. The 24h screen included assays to determine the effects of PFAS ranging from 50-500µM on proliferation, cell viability, and mitochondrial function. Doses were selected based off assay optimization due to limitations imposed by selecting human-relevant doses for chemicals for which human exposures have yet to be defined. Of 33 PFAS screened, 56% showed effects on proliferation, 36% on mitochondrial function, and 39% on cell viability. To determine a potential mechanism of FGR for PFOA and evaluate whether its replacement, GenX, has similar effects to human placental cells, we performed a dose-response screen to determine whether GenX had increased placental and weights (18% and 22% respectively), decreased fetal weights (21% and 5% respectively), decreased fetal length (6% and 0.6% respectively) and decreased fetuses/placenta ratios (32% and 21% respectively).
These data suggest both PFOA and GenX adversely affect the feto-placental unit, possibly via diverging mechanisms where PFOA appears to affect both the fetus and placenta, whereas GenX appears to primarily impact the placenta. These findings implicate GenX as a potential public health threat rather than a safe alternative.

1720 Effect of Lifestyle-Based Lipid-Lowering Interventions on the Relationship between Circulating Levels of Per- and Poly-Fluorinated Chemicals and Serum Cholesterol
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Exposure to certain per- and polyfluoroalkyl substances (PFASs) have been shown to positively associate with total and/or low density lipoprotein cholesterol (LDL), but data from cross-sectional epidemiological studies are limited. People residing in Kentucky and surrounding states may face higher exposures due to nearby manufacturing. We examined the association between 6 PFASs and circulating total and LDL cholesterol levels in Kentucky residents undergoing a cardiovascular risk reduction clinical study. We developed high throughput plate-based extraction and LC-MS analysis methodologies to quantify PFASs in 350 individuals. Plasma and demographic information was collected and bivariate statistics and logistic regression modelling were used to examine associations of circulating PFASs and cholesterol levels. PFOS, PFOA, FPhxS, and FFHxA, significantly decreased post intervention (13.25-11.12 ppm, 2.20-1.99 ppm, 0.96-1.01 ppm, and 0.40-0.14 ppm respectively). Interestingly, PFOS as well as the combined sum of 6 compounds (Total PFAS), significantly positively associated with total cholesterol in post-intervention samples (Pearson correlation coefficient 0.132; p=0.0241, 0.115; p=0.0497 respectively). After adjustment for multiple covariates including gender, BMI, smoking, race, education level, and age; Total PFAS was still significantly positively associated with total cholesterol (p=0.021). In general, PFAS levels in this population were similar to NHANES levels, although PFOS exposures appeared to be increased. This work adds to a growing body of knowledge associating circulating PFAS and total cholesterol levels in humans.

1721 Targeted Gene Expression Assays Reveal Markedly Different Gene Expression and Lipid Accumulation Profiles for Perfluorooctyl Acid (PFAA) Mixtures Compared to Single PFAA Treatment in Cryopreserved Human Hepatocytes
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Perfluoroalkyl acids (PFAAs) are a family of fully-fluorinated chemicals that are used in fire-fighting foams and as well as many heat-, stain- and water-resistant materials in a variety of applications. These chemicals, such as perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) cause liver steatosis in animal studies. It is unclear whether human exposure to PFAAs causes liver steatosis. To date, the developed method will have broad applications towards understanding the toxic mechanisms of PFASs. The developed method will have broad applications towards understanding the toxic mechanisms of PFASs. The developed method will have broad applications towards understanding the toxic mechanisms of PFASs. These data suggest both PFOA and GenX adversely affect the feto-placental unit, possibly via diverging mechanisms where PFOA appears to affect both the fetus and placenta, whereas GenX appears to primarily impact the placenta. These findings implicate GenX as a potential public health threat rather than a safe alternative.

1722 Perfluorooctanesulfonic Acid (PFOS) Is Selectively Neurotoxic to Dopaminergic Neurons in C. elegans
S. R. Sammi, and J. R. Cannon. Purdue University, West Lafayette, IN.

Perfluorinated compounds (PFCs) have been widely utilized as stain repellents, flame retardants, coating additives in nonstick cookware and in the food packaging industry. Given the long environmental and biological half-lives, bio-accumulative and surfactant properties, PFCs are common soil and water contaminants, detectable in the blood of >99% of individuals. PFC exposure has been linked to a number of adverse health outcomes, including cancer risk, altered lipid homeostasis, hepatotoxicity, and developmental abnormalities. However, the neurotoxicological effects of PFCs have not been sufficiently studied. Given known effects on calcium homeostasis and oxidative stress, we hypothesized that PFCs would be neurotoxic and that dopaminergic neurons would be especially sensitive. We tested a number of PFCs for neurotoxicity in nematode models: perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), 1H,1H,2H,2H-Perfluoro-1-decanol, heptaffluorobutyric acid (HFBA). PFOS exposure (within the range 75 to 150 ppm for 72 hrs) was found to produce neurotoxicity in C. elegans in dopaminergic, serotonergic and GABAergic, and cholinergic neurons as evidenced by overt and quantifiable morphological changes, including loss of processes and swelling of the soma. Dopamine neurons were especially sensitive, exhibiting evidence of neurotoxicity at doses below the toxicity threshold for other neurotransmitter systems (as low as 75 ppm). PFOs treatment also resulted in loss of mitochondrial viability, altered α-synuclein aggregation, and elevated reactive oxygen species. PFOs-treated worms showed dopamine-dependent deficits but not acetylcholine-dependent changes in functional assays. Surprisingly, all other tested PFCs were not neurotoxic at tested doses (HFBA (107 - 1070 ppm); PFOA (10 - 100 ppm)), potentially indicating that the sulfonate hydroxide may be especially important to PFC-induced neurotoxicity. Our results suggest that PFOS is selectively neurotoxic to dopamine neurons and, to our knowledge, the first example of a potential relevance to Parkinson’s disease. Furthermore, alternative and replacement PFCs carrying the sulfonyl hydroxide functional group should also be studied for neurotoxicity.

1723 Development of a Chemical Proteomics Method for the Enrichment and Identification of Poly- and Per-Fluorinated Bound Proteins
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The persistence, bioaccumulation and toxicities of Poly- and Per-fluorinated compounds (PFCAs) are of great concern. Specifically, some PFCAs cause cancer, such as fluorotelomer alcohols (FTOHs) have been shown to co-valently bind to proteins; cysteine reactivity has been suggested to be one of the primary toxic pathways for these PFCAs or their bioactive metabolites. While previous studies have been conducted to investigate their toxicities, exact toxic mechanisms remain unclear. In this study, we developed a chemical proteomics method to identify the protein targets of cysteine reactive FTOHs. Our method was developed initially using a fluorosulfosuccinimidyl tagged bovine serum albumin before analyzing liver proteins extracted from 8.2 perfluorooctanoic acid (PFOA) exposed rats. PFOA-bound peptides were enriched by filtering tryptic digest via solid-phase fluoruous extraction (SPFE). One-step enrichment yielded more than 80% of PFC-bound peptides while removing more than 90% of native peptides. We report a 30% increase in the number of detected PFC-bound peptides when compared to traditional proteomic sample workups. PFOA-bound peptides were found to be more hydrophobic than native peptides, and PFOA-bound peptides tended to elute within a narrow 5 minute with a classic water/acetoniitrile nano-LC gradient (35% acetonitrile maximal effective mobile phase). Poor chromatographic resolution greatly reduced data-dependent acquisition of PFC-bound peptides. Consequently, we optimized a new gradient to further increase chromatographic resolution of PFC-bound peptides. With our improvements, PFC-bound peptides were eluted over a 120-minute gradient. This further increased the sensitivity of our chemical proteomics method, and the number of PFCAs-bound peptides increase by two folds. By carefully evaluating the mass spectra, covalent cysteine amino acid residues were identified as the primary binding site of 8.2 PFOA. Thus, 8.2 PFOA-cysteine modifications were added to the list of MaxQuant modifications to enable automatic proteome database searching. Thus, we have developed the first chemical proteomics method to directly identify protein targets of PFAAs. The developed method will have broad applications towards understanding the toxic mechanisms of PFAAs.
Hexafluoropropylene oxide dimer acid (HFPO-DA, aka GenX) is a member of the per- and polyfluoroalkyl substances (PFAS) chemical class and a high-profile contaminant of emerging concern. Elevated levels of HFPO-DA have been detected in multiple environmental matrices and treated drinking water in the United States indicating widespread use and ecological receptors. Our goal was to characterize the potential mammalian toxicity of HFPO-DA from oral administration during gestation to maternal and pre/postnatal rats. Initially, we dosed Sprague-Dawley rat dams daily via oral gavage with 1-500 mg HFPO-DA/kg/d during the critical window for male reproductive tract development (gestation day (GD) 14-18). We evaluated fetal testis testosterone production, expression of PPAR signaling pathway genes in maternal and fetal livers, maternal serum clinical chemistry, reproductive development of F1 animals, and internal dosimetry in maternal serum and fetal plasma. HFPO-DA exposure during gestation (GD 14-18) did not affect the fetal testis, but did produce significant dose-responsive increases in maternal liver weight (≥26.5 mg/kg), reduced maternal serum thyroid hormone and lipid profiles (≥30 mg/kg), and highly upregulated gene expression related to PPAR signaling pathways in maternal and fetal livers (≥1 mg/kg). A low sample size (n=3 per dose) pilot postnatal study at 125 mg/kg/d was largely negative for adverse effects but indicated potential reductions in female body weight and reduced weights of male reproductive tissues in F1 animals. However, a follow-up postnatal study covering a broader dosing interval (GD8 - postnatal day 3), greater sample size (n=5 per dose), and greater dose range (10-250 mg/kg/d) resulted in significant, dose-responsive neonatal mortality at ≥62.5 mg/kg/d and reduced body weight of surviving pups at all doses (≥10 mg/kg/d). This study is ongoing with evaluation of adult F1 animals that were exposed in utero. Overall, HFPO-DA exposure produced multiple adverse effects similar to previously published PFAS toxicity evaluations, such as PFOS and PFDA, and in vivo toxicokinetics seems to play a major role in the relative potency of these compounds for producing effects. The views presented here do not necessarily reflect the views or policies of the US EPA.
1728 The Development of a Database for Herbal and Dietary Supplement Induced Liver Toxicity
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The growing use of herbal dietary supplements (HDS) in the United States provides compelling evidence for risk of herbal-induced liver injury (HILI). Information on HDS products was retrieved from MedlinePlus of the U.S. National Library of Medicine and the herbal monograph of the European Medicines Agency. The hepatotoxic potential of HDS was ascertained by considering published reports. Other relevant data were collected from governmental documents, public databases, web sources, and the literature. We collected information for 296 unique HDS products. Evidence of hepatotoxicity was reported for 67, that is 1 in 5, of these HDS products. The database revealed an apparent gender preponderance with women representing 61% of HILI cases. Culprit hepatotoxic HDS were mostly used for weight control, followed by pain and inflammation, mental stress, and mood disorders. Commonly discussed mechanistic events associated with HILI are reactive metabolites and oxidative stress, mitochondrial injury, as well as inhibition of transporters. HDS-drug interactions, causing both synergistic and antagonizing effects of drugs, were also reported for certain HDS. The database contains information for nearly 300 commonly used HDS products to provide a single-entry point for better comprehension of their impact on public health.

1729 Deep vs. Conventional Machine Learning Models for Predicting Ames Mutagenicity
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Deep machine learning methods have emerged as powerful techniques to extract knowledge from large amounts of data. As expected, they are rapidly making their way in the field of QSAR modeling. In this work, a big set of chemicals (17,008) with their Ames mutagenicity outcomes was utilized to build deep neural network models and evaluate their predictive performance. A set of 1,942 chemicals were used as an external test set. Long short-term memory (LSTM) recurrent neural networks were used for this purpose. In addition, a conventional logistic regression QSAR model composed of molecular fragment descriptors was constructed for comparison. Both LSTM and conventional fragment-based models showed excellent performance; however, the LSTM model fared marginally better. Deep learning (LSTM) model: Sensitivity = 87%, Specificity = 88%, Coverage = 100%; Conventional fragment-based model: Sensitivity = 83%, Specificity = 91%, Coverage = 100%. In order to facilitate interpretation of the prediction results, the ability of the LSTM models to identify mutagenicity alerts in test chemicals was also investigated. This became possible by adding an attention mechanism in the LSTM network which highlighted parts of SMILES code directly that are relevant to the mutagenicity of a test chemical. The conventional model is highly interpretable because it is composed of fragment-based alerts that were mined directly from the training data. We anticipate that deep learning QSAR models will be highly valuable and accurate for the research and regulatory decision-making communities, particularly as the volume of available high-quality Ames test data increases.

1730 Ensemble QSAR Modeling to Predict Multispecies Fish Toxicity Points of Departure
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QSAR modeling is prioritized in the thousands of chemical substances for which no ecological toxicity data is available. We pulled experimental results from the US Environmental Protection Agency’s ECOTOX database and the European Chemical Agency’s database to build a large data set containing in vivo test data on thousands of chemical substances and hundreds of species of fish. This data set was used to create QSAR models to predict two types of potential points of departure (POD): acute LC₅₀s (median lethal concentration) and endpoints comparable to the NOEC (no observed effect concentration) for any duration and measured effect. In addition to molecular descriptors and physiochemical property predictions, the QSAR models used study covariates, such as species and exposure route, as features to maximize accuracy when combining multiple data types. A novel method of substituting taxonomy groups for species dummy variables was introduced to allow the model to generalize to other species. A stacked ensemble of three machine learning methods—random forest, gradient boosted trees, and support vector regression—was implemented to increase accuracy and minimal feature selection. The models predicted LC₅₀s and NOECs within one order of magnitude 81% and 76% of the time, respectively, and had root-mean-square-errors (RMSEs) of roughly 0.83 and 0.98 log₁₀(mg/L) respectively. Benchmarks indicated that the prediction accuracy was improved beyond the 95% confidence intervals of existing models. This abstract does not necessarily represent US EPA policy.

1731 Computational Association of Permethrin Exposure and Asthma in California Agricultural Counties

Asthma rates in California have shown an increase in counties with extensive use of the pesticide permethrin. Notably the surrounding agricultural area of Imperial County reports significant asthma related admissions to the emergency room every year. Biomonitoring studies using urinary levels of the metabolite 3-phenoxybenzoic acid (3-PBA) in Monterey County, the second highest permethrin usage county in California, and in military personnel wearing permethrin treated uniforms have confirmed the exposure-bioaccumulation correlation. In separate studies, the most frequent users of permethrin were more likely to report wheeze, both allergic and non-allergic during medical exams, and there exists suggestive evidence of wheeze associated with permethrin exposure among children. This study utilizes online databases, docking software, data networks, predictive molecular modeling software and literature to identify a potential chemical-gene-disease link between permethrin exposure and asthma. Our results support a positive chemical-gene interaction between the estrogen receptor (ER) and permethrin. Additionally, docking software confirmed significant binding affinity between ESR1 and permethrin. These findings suggest that the typical inflammation preceding asthmatic symptoms can be induced by ESR1 ligand binding via a spectrum of immune functions, including adhesion, migration, and antibody and cytokine production. Induction of these pathways can be attributed, in part, to ligand binding of estrogen receptors found on the surface membrane of various immune cells within the alveolar and pleural cavities. This activation has been implicated as a main regulator of the lung’s inflammatory response, a hallmark of asthma. Pathway analysis suggests LFA-1 binding to ICAM leading to upregulation of pro-inflammatory IL-5 and IL-4 via MAPK induction plays a part in this chemical-disease linkage. Consequently, an increased presence of permethrin, a viable ESR1 ligand, may be contributing to the higher incidence of asthma in Imperial County, California via activation of the ESR1 pathway. It is not known if single nucleotide polymorphisms (SNPs) in the estrogen receptor alpha gene might identify a more susceptible risk population.

1732 Analyzing ToxCast Data Using Nebula (Neighbor-Edges Based and Unbiased Leverage Algorithm)
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Most so called “big datasets” are incomplete, with varying degrees of scarcity. They pose special difficulties across the board for traditional machine learning and classification algorithms. Yet, the future of big data is now, equating to some urgency in finding approaches for overcoming the challenges of analyzing sparse datasets. In this study we developed the Neighbor-Edges Based and Unbiased Leverage Algorithm (Nebula) to tackle sparse big data. The US Environmental Protection Agency’s (US EPA) ToxCast project evaluated a diverse set of chemicals, including both environmental chemicals and drugs, using a broad panel of high-throughput in vitro assays. ToxCast data have been studied to characterize the toxicological profiles of environmental chemicals. However, the dataset has a high degree of missing elements and thus is sparse. To warrant full utilization of ToxCast dataset generated from a huge US EPA investment in risk assessment of chemicals, novel and comprehensive analyzing the dataset is needed. Therefore, as a test, we applied Nebula and modularity analysis for ToxCast data. We found that the chemical-assay network could be decomposed into seven densely connected modules based on its topological properties. Moreover, each of the seven modules was associated with different set of adverse outcome pathways (AOPs) as well as chemical structural descriptors. Leave-one-out cross validations showed a high consistency between experimental AC₅₀ values from ToxCast and predicted AC₅₀ values from Nebula. Our study demonstrated Nebula to be an efficient algorithm for analyzing sparsely populated big data and, thus, useful in the big data era. Our results also indicated ToxCast data could be used for toxicologically profiling chemicals that have not been assayed in ToxCast to assist risk assessment of chemicals.
In animal-free risk assessments, in vitro effect concentrations need to be related to external e.g. oral doses (in vivo-in vitro extrapolation, IVIVE). In our investigations, we used a simple 1 compartment model as well as a 8-compartment PB-P model for the rat as tools for reverse dosimetry and applied these models for 10 potential endocrine disruptors (e.g. Bisphenol A (BPA), Fenamilo (FEN), 17α- Ethynylestradiol (EE), Asetaminophen (APAP), Caffeine (CAF), Ketoxonazol (KET), Flutamide (FLU), Genisint (GEN), Methyltestosterone (MTT), Trenbolone (TRE)). The compounds have been tested in the Yeast Estrogen Screening (YES) or Yeast Androsten Screening (YAS) assays for the estrogen and androgen receptor binding, as well as the H295R assay (OECD test guideline no. 456) for potential interaction with steroidogenesis. Postulating comparable concentration-response ratios of these effects in the applied in vitro systems and the in vivo environment, lowest observed effect concentrations (LOECs) from these assays were extrapolated to oral doses (LOELs) by reverse dosimetry. The predicted LOELs were then compared to the LOELs actually observed in corresponding in vivo studies (YES/YAS assay versus uterotrophic or androsten receptor assay and steroidogenesis assay versus pubertal assay or generation studies). Reverse dosimetry resulted in estimated oral LOELs for the rat in the same order of magnitude than in literature described in vivo-derived LOELs for 7, BPA, FEN, APAP, CAF, KET, FLU, MTT and 6, BPA, FEN, APAP, CAF, KET, GEN out of 10 substances for the applied 1 compartment and the applied 8-compartment PB-P model. In conclusion this demonstrates the applicability of the applied concept in general, the need for its future optimization and allows us to derive questions on what we need to know to further optimize these calculations.

In vitro to In Vivo Extrapolation of High-Throughput Screening Assay for Thyroperoxidase Inhibition

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High-throughput (HTP) assays are being used to rapidly assess the potential of chemicals to interfere with the thyroid system. Amplex-UltraRed (AUR)-thyroperoxidase (TPO) inhibition assay is a rat microsomal HTP assay developed to screen the thyroid synthesis enzyme, TPO. Although AUR-TPO assay has accelerated chemical testing, translation of in vitro outputs to in vivo measures of thyroid dysfunction remain an obstacle to their use in risk assessment. Our goal was to explore in vitro to in vivo extrapolation (IVIVE) approaches to aid in interpretation of AUR data. Adult rats were exposed to varying doses of two known TPO inhibitors, methimazole (MMI) and 6-propyl-thiouracil (PTU) via drinking water for 4, 7, or 14 days. We first compared in vitro TPO inhibition to ex vivo TPO inhibition using thyroid gland microsomes prepared from the glands of PTU and MMI exposed animals. A second approach was to introduce chemical and glandular hit rates obtained in vitro to a one-compartment pharmacokinetic (PK) model to derive an in vivo IC50. Serum and gland PTU and MMI levels increased in a dose- and time-dependent manner. Similarly, PTU and MMI reduced serum and gland triiodothyronine (T3) and thyroxine (T4) and increased serum thyroid stimulating hormone (TSH) in a dose- and time-dependent manner. Estimates of IC50 for TPO inhibition from the ex vivo study ranged from 108-169 µM and were higher than those from in vivo exposure in microsomes from naïve glands (IC50=0.1-1.2 µM). Ex vivo estimates were well correlated with serum T4 at 4-day exposures but were inconsistent at later timepoints. The second approach to IVIVE incorporated the measured values of thyroid gland T4, MMI, and PTU into a PK model. Excellent fits of the 4-day PTU data were achieved with an estimation of an in vivo derived TPO inhibition of 6.2 µM, a value 5-fold higher than the in vitro estimate of 1.2 µM. Assessment of 7- and 14-day exposures for PTU and for MMI are underway. In summary, ex vivo measures of TPO inhibition using the AUR assay were highly correlated with serum thyroid hormones at short exposure durations, but were inconsistent at longer exposures, and IC50s were much higher than in vitro estimates. PK derived in vivo estimates of TPO inhibition with short exposures to PTU were more closely aligned to in vitro estimates. Our results provide an in vivo based anchor to aid in interpretation of HTP outputs of thyroid disruption. Does not reflect US EPA policy.

Utilization of the Ascentos LIMS and TranSEND to Streamline the Collection and Compilation of Data for Send Dataset Creation

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Ascento’s is an extensive, full-scale Laboratory Information Management System (LIMS) that provides a structured and regulated method to collecting data in a non-clinical environment. A companion system to Ascento’s is TranSEND™, which is used to compile the data that was collected in the LIMS system and export it in a Standard for Exchange of Nonclinical Data (SEND) data set. The objectives of this poster are to display the processes and ease of converting data collected in a LIMS system, as well as data that was received from outside sources that do not utilize a LIMS system, to an acceptable and approved SEND data set. Ascento’s enables the user to correctly populate the study protocol and schedule activities in a way that complies with the regulations on SEND submission. One major benefit of using Ascento’s in conjunction with TranSEND™ is that Ascento’s allows the user to build glossaries based on Controlled Terminology. This specific feature virtually diminishes the need to map terms in TranSEND™, which ultimately significantly reduces the man hours required to set up a dataset. TranSEND™ imports and unites study specific Ascento’s data, external consultant data files, and data sets that were created using compounds that were provided by PDS. The complete dataset is exported and includes a validation report that notifies the user of any rejects, errors, warnings, and notices within the set and also a pre-populated Non-Clinical Study Data Reviewer’s Guide (nSDRG) that is user friendly for completion.
1737 Retrospective and Prospective Case Studies to Accelerate the Pace of Chemical Risk Assessment

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Use of high-throughput, in vitro bioactivity data in setting a point-of-departure (POD) has the potential to accelerate the pace of human health risk assessments by chemical prioritization. Advancement toward this goal requires confidence in in vitro bioactivity data, in conjunction with high-throughput toxicokinetic information, can be used to estimate administered equivalent doses at or below the PODs from traditional animal studies. Further, hazard and exposure predictions, combined as a bioactivity-exposure ratio (BER) for use in risk-based prioritization, should be evaluated. In this work we describe two efforts of the Accelerating the Pace of Chemical Risk Assessment initiative, a consortium of international regulatory scientists, both with the same primary objective: to elucidate whether a POD derived from in vitro bioactivity would be a conservative estimate of traditional POD estimates, and if the BER is a useful prioritization metric. In the first project, we describe the outcome of a retrospective case study of 448 chemicals with high-throughput predictions of bioactivity, reverse dosimetry, and exposure, as well as traditional hazard information. For 92% of these chemicals, a POD derived from new approach methodologies (POD_{NM}TM) was a conservative prediction for the traditional POD (POD_{informed}) value. High-throughput exposure predictions were greater than the POD_{NM} for 26/448 chemicals, with BERs of less than zero, indicating higher priority for further investigation. The second, prospective study involves generation of Nam data for 200 chemicals to prioritize 20 chemicals for 90-day repeat dose testing in rats using a combination of the BER and bioactivity-based flags. Together these case studies enable regulatory scientists from different international contexts to develop efficient approaches for chemicals management, while possibly reducing the need for animal studies. This work demonstrates the feasibility, and continuing challenges, of using bioactivity and exposure NAMs in screening level safety assessment. This abstract does not necessarily reflect US EPA policy.

1738 An AOP-Based Ontology for Spina Bifida Caused by Disturbance in Retinoid Acid Signaling

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The retinoic acid (RA) pathway serves as a prototype for adverse outcome pathway (AOP) elucidation for developmental defects. The biology of the RA pathway is used for the construction of an AOP for neural tube closure defects. The ontology describes an AOP network incorporating retinoid homeostasis and putative molecular initiating events in chemical teratogenesis. Basic elements in the ontology are subjects (enzymes, receptors, cell types) and their quantitative relationships (response-response relationships), forming a network of biological interactions to be mapped to a vulnerable developmental window for e.g. teratogen-induced spina bifida. We have searched the available literature data using text-mining tools to map known molecular interactions, genetic signals and responses that: (a) play a crucial role in cellular differentiation; (b) establish anterior-posterior gradients (FGF and RA signaling) and dorsal-ventral gradients (zinc factors [Zic] and BMP signaling) for regional specification. Molecular initiating events important for RA balance (like CYP26 enzymes and RALDH2) potentially affected by xenobiotic compounds (based on high-through-put screening data), were connected with toxicological data on the development of posterior neural tube defects. Ultimately, this network can be dynamically modeled in silico, providing an integrated computational systems model allowing toxicity predictions at the level of adverse outcomes in the intact individual. A battery of cell-based in vitro assays can be used to monitor the critical rate-determining steps in the network, providing a tiered testing strategy to collect data feeding into the systems model. Integrating the dynamic model with information from exposure and kinetic models allows quantitative hazard and risk assessment while avoiding animal testing. The views presented in this abstract do not necessarily reflect current or future opinion or policy of the US Environmental Protection Agency and the US Army Corps of Engineers.

1739 Characterizing Developmental Toxicity through Pluripotent Stem Cell Assays and the ToxCast Library

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Predicting and characterizing potential human teratogenic compounds remains a challenge as toxicology moves towards new approach methodologies (NAMs) for hazard identification. Utilizing pluripotent stem cells, in conjunction with high throughput screening technologies, has the potential to aid developmental hazard prioritization for large numbers of chemicals and lessen the strict reliance on costly and time-consuming animal methods. To investigate this potential, ToxCast chemicals were profiled for developmental toxicity in two embryonic stem cell assays: the human pluripotent H9 stem cell-based devTOX quickPredict platform from Stemina (STM) and the mouse embryonic stem cell (mESC) adherent assay. To test model performance, the STM platform was tested using two sets of compounds: a benchmark set of 42 compounds (BM42), of which 26 are classified as developmental toxicants and a set of 432 ToxRefDB compounds (TR432), of which 187 had reported evidence of developmental toxicity from prenatal rat or rabbit developmental toxicity tests (lowest effect level ≤200 mg/kg/day). When compared against the BM42 set, the STM platform had a model performance of 78.3% accuracy (0.65 sensitivity, 1.0 specificity, 0.79 f1) and against the TR432 compounds, STM had 61.3% accuracy (0.31 sensitivity, 0.84 specificity, 0.41 f1). Using the mESC adherent assay, chemicals from the TR432 compound set demonstrated a model performance for the mESC platform of 84% accuracy. To investigate utilizing pathway-based predictions for developmental toxicity screening, 32 enzymatic and receptor assays in the ToxCast NovaScreen data-set (NVS) were trained on the STM hit call data to characterize biological pathways associated with STM positive and STM negative responses. Including NVS-derived pathway data with the STM platform increased model accuracy against the BM42 set to 83.3% (0.77 sens, 0.88 spec, 0.83 f1) while accuracy against the TR432 set was 60% (0.55 sens, 0.64 spec, 0.54 f1). These findings have set the stage for identifying and developing new approach methodologies based on in vitro data and in silico models for prenatal developmental toxicity. This abstract may not reflect US EPA policy.

1740 Combining Structural Alerts and Machine-Learning Algorithms to Predict Human Molecular Initiating Events

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Molecular Initiating Events (MIEs) are important chemical-biological interactions that start Adverse Outcome Pathways (AOPs). MIEs provide good targets for computational modelling as they are right on the boundary between biology and chemistry, and any computational models produced for them do not skip over large amounts of biological complexity, compared to trying to predict responses at an organ or organism level. We have developed in silico models for the prediction of important human MIEs for use in risk assessment using chemical understanding. The first of these approaches uses structural alerts, chemical fragments of known binders, to predict MIEs. Open source data from ChEMBL and ToxCast was used, providing positive and negative data points for each human MIE. Structural alerts were then automatically constructed using a computational algorithm developed in KNIME, which used maximal common substructure searches and Bayesian reasoning to choose the best structural alerts and build the best models for each biological target. Models for human MIEs including G-protein coupled receptors, nuclear receptors, enzymes, ion channels and transporters have been constructed. This includes important biological targets including Androgen and Glucocorticoid Receptor disruption which can lead to developmental toxicity, HERG Channel blocking which can lead to cardiac toxicity, and cyclooxygenase inhibition which can lead to reproductive dysfunction. The second of these approaches involves the use of machine learning algorithms fed with chemical information to predict MIEs. The same data sources are used to train neural networks, resulting in a high level of predictivity. Chemical and biological similarity calculations using the neural networks have also been performed to better understand how these artificial intelligence approaches provide predictions. Most of the models developed using these methodologies predict at accuracy levels above 80%, and a number predict above 90%, showing extremely good performance using holdout cross-validation. Combining these orthogonal in silico approaches provides further confidence in their predictions and a larger impact for computational toxicology. The in silico predictions of these MIEs is extremely important for the future of AOP based risk assessment. By incorpo-
1741 Detection of Chemical Bioactivity Using In Vitro Phenotypic Profiling
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High-throughput (HT) in vitro assays have the potential to accelerate the prioritization and assessment of chemicals with little or no human safety data. However, it remains a challenge to balance the sensitivity and specificity of HT in vitro assays to identify relevant chemicals. Here, we present a new approach for bioactivity detection called HT In Vitro Phenotypic Profiling for Toxicity Point-of-Departure detection (HIPPTox-POD). We used automated image processing to measure 165 phenotypic features from three human cell lines: BEAS-2B (bronchial epithelial cells), HepG2 (hepatocarcinoma cells), and HK2 (proximal tubule cells). The diverse phenotypic features and cell lines allow us to detect perturbations to different biological pathways. Then, we used machine learning to detect perturbed cells, and estimated concentration-response curves (CRC) based on changes in cellular phenotypes. We tested 64 blinded chemicals, including phthalates, phenols, conozoles, and other chemicals, provided by the US EPA. We obtained reproducible CRCs for 47 chemicals, with only 13 chemicals failing the effectiveness concentration at 10% of the curve (POD_{10\%}). Two distinct clusters of chemicals were observed: eight with high POD_{10\%} values (“inactive”) and 39 with low POD_{10\%} values (“active”). We also examined the 5th-percentile of the active concentrations at 50% activity across all the ToxCast assays (POD_{5\%}). For the same chemicals, but no clear clustering of the chemicals could be found. Interestingly, when compared to the 5th-percentile of the NOAELs/LOAELs of these chemicals from ToxRefDB (POD_{5\%}), we found that the inactive chemicals have higher POD_{5\%} than most of the active chemicals. Furthermore, POD_{HIPPTox} is positively correlated to POD_{ToxCast} (Spearman’s rank correlation coefficient = 0.691, P = 1 x 10^{-11}). Also, POD_{HIPPTox} of chemicals with POD_{ToxCast} = 1 μM, but no significant correlation was observed for active chemicals with POD_{ToxCast} ≤ 1 μM. Our results show that HIPPTox and Toxcast produce similar rankings of chemicals in 64% of the active chemicals. Toxcast is more sensitive in detecting bioactivity, but HIPPTox-POD is more specific and may identify chemicals with weak or no bioactivity under standard animal tests. Therefore, HIPPTox-POD may be used together with ToxCast to provide a more balanced screen of chemicals. This abstract does not reflect US EPA policy.

1742 A General Approach to Model Errors of Machine-Learning Prediction of Chemical Toxicities
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A known limitation of conventional machine-learning methods for quantitative structure-activity relationship (QSR) studies of molecular activities is that the prediction error for a molecule depends on the similarity or “distance” between that molecule and the molecules in the training set. The concept of applicability domain (AD), which is used to gauge the reliability of QSAR predictions, assumes that a prediction is reliable if a molecule is within the AD and unreliable if it is not. This poses a conundrum because, although we are most interested in predicting the activities of novel molecules, such molecules are most likely outside the AD and, therefore, we have the least predictive confidence. Recently, deep neural networks (DNNs) have become the tool of choice in artificial intelligence, owing to their ability to outperform all other machine-learning methods. However, it is unclear whether they provide similar performance and reliability in predicting chemical toxicity measurements. To address this question, we recently analyzed DNN predictions of chemical toxicities in detail. The results showed a similarity-dependent degradation of prediction accuracy comparable to other machine-learning methods, suggesting the need to implement AD measures for DNNs as well. In this study, we developed a molecular Tanimoto distance-based AD metric suitable for any machine-learning method. We define this new metric as the sum of the distance-weighted contributions (SDC) of all training molecules, where all molecules in the training set contribute, but the contribution of each is weighted by how similar the query molecule is to each molecule in the training set. Using many chemical toxicity datasets and machine-learning methods, we showed that the correlation between the prediction error and the SDC is stronger than that between the prediction error and other AD metrics. This allowed us to construct a root mean squared error (RMSE) prediction model based on SDC, and thereby quantitatively estimate prediction errors. The strength of the new metric is its ability to accurately estimate errors for molecules outside the AD of a model. The metric thus gives us the ability to know how accurately we predict the truly unknowns.

1743 Hallmark Gene Set Annotation for NTP Toxicogenomic Studies
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The Hallmark Gene Sets were originally generated by Liberzon and colleagues to represent defined biological processes and coordinated expression patterns. The gene sets were identified through computational and manual curation methods to identify overlapping gene sets in Molecular Signatures Database (MSigDB) collections retaining genes that display coordinated expression. The development of this gene set helped facilitate more robust Gene Set Enrichment Analysis (GSEA) results. The National Toxicology Program (NTP) toxicogenomics program uses short term exposures in rodents to assess the effects of test article exposure on gene expression. Changes in gene expression can be used to model the toxic effects of the test article. This can be done by comparing the effects with other substances in other databases and analysis tools. To enhance the NTP analysis of genomic effects, the Hallmark Gene Sets have been manually curated and refined for relevance to the NTP endpoints. The current work focuses on the EIF Bio platform to identify correlations between Hallmark Gene Sets and genes upregulated or downregulated in rodent liver. Using this information, a mechanistic interpretation was developed for the Hallmark Gene Sets that can be used to enhance GSEA for NTP studies. The Chemical Effects in Biological Systems (CEBS) database is a public resource for NTP toxicology testing data. The annotated Hallmark Gene Set can be reviewed, searched and downloaded from the CEBS NTP Data Collections application (https://manticore.niehs.nih.gov/datasets).

1744 A Novel Evidence Integration Framework: Incorporating Human, Animal, and Mechanistic Data for Causal Inference
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This novel Evidence Integration Framework (EIF) provides a method for synthesizing data from comprehensive, systematic, quality-based assessments of the epidemiological and toxicological literature, including in vivo and in vitro mechanistic studies. The EIF organizes data using both a disease-based and mechanism-based scheme to support evidence synthesis. The disease-based scheme uses the evidence of human health outcomes studied in the best quality epidemiological literature to organize the toxicological data according to authors’ stated purpose, with the pathophysiology of the disease determining the potential relevance of the toxicological data to the human health endpoint. The mechanism-based scheme organizes the toxicological data based on the proposed mechanisms of effect and mechanistic data supporting key events leading to each toxicological endpoint, with corroborating epidemiological data providing a bridge to human health effects. In addition, the data are organized into major and minor domains, to assist in characterizing the uncertainty of the relevance of the toxicological data to human health. The EIF includes a method to cross-classify and describe the concordance of the lines of evidence, to further characterize the uncertainty of their connection and thereby to draw causal inferences. An initial application of the EIF Bio platform focused on the integration of evidence related to non-acute exposure to nicotine and cancer and identified signals that there may be a relationship between the two. The evidence is largely driven by weak evidence from the epidemiological literature pertinent to smokeless tobacco use, and toxicological data that can be best classified as minor or supportive in nature. The EIF thus identifies a direction for future research, i.e., investigating the meaning of these signals and determining whether integrating the epidemiology with the toxicology might have resulted in conclusions that were not expected and require further investigation.
The rapidly increasing inventory of publicly available in vitro high-throughput screening (HTS) assay data is facilitating the development of computational approaches for chemical hazard assessment. With HTS assays for more than 1,000 endpoints, the United States Environmental Protection Agency’s ToxCast program provides insight into a wide variety of molecular and cellular targets. While ToxCast data is annotated to include information about technology platform, assay design, and gene target (where appropriate), it remains a challenge to place assay outputs into a toxicological context. Here we present a framework for mapping ToxCast HTS assay endpoints to toxicological adverse outcomes, moving beyond assay annotations to molecular biological targets to provide a more robust assay grouping schema. To date, 168 ToxCast assay endpoints have been manually mapped to “acute systemic toxicity” by linking them to distinct modes-of-action (MoA) known to be relevant to acute systemic toxicity. Acute systemic toxicity MoAs rich in ToxCast data include mitochondrial inhibition (20 assay endpoints), altered ion flow (23 assay endpoints), and oxidative stress (27 assay endpoints). Likewise, 154 assay endpoints have been mapped to “developmental toxicity”, for which MoA groupings include neural crest cell disruption (26 assay endpoints), endocrine disruption (49 assay endpoints), and vascular disruption (23 assay endpoints), among others. To demonstrate the utility of MoA mapping for toxicity outcomes, we present a case study using the ToxPi prioritization approach, which leverages weighted relationships across various MoAs to yield insight into the potential of a chemical to elicit developmental toxicity. This approach provides a linkage of in vitro HTS assays to toxicological MoAs, which can facilitate chemical prioritization (i.e., with ToxPi), inform predictive modeling, or even aid in developing the foundation for adverse outcome pathways. This project was funded in whole or in part with federal funds from the NIEHS, NIH under Contract No. HHSN275201500010C.

Analysis of groundwater in Gela, Italy revealed significant contamination from a local industrial site. One of the chemical compounds, ethylene dichloride, was found to be present in the highest concentration per legislative allowed value. Gela has also reported an unusually high incidence of hypospadias, a congenital disorder characterized by the abnormal location of the urethral opening, with a prevalence nearly double that of Italy’s and one of the highest in Europe. This study investigates the computational association between ethylene dichloride exposure in Gela and hypospadias. Online databases, docking software, and literature were used to identify a potential genetic link between the two. The HSD3B2 gene, which is expressed in the gonads, and the HSD3B1 gene, expressed in the placenta, were of interest, since both are crucial in steroid biosynthetic processes associated with hypospadias. Biomonitoring and teratogenic studies revealed that ethylene dichloride crosses the placental barrier and accumulates in both placental and fetal tissues for approximately 7 days. Ethylene dichloride was hypothesized to act in parallel in both the mother’s placenta, via ATP3 and HSD3B1 mRNA upregulation, and the fetal gonads, via HSD3B2 mRNA downregulation. Based on the comparative analysis, a molecular initiating event for ethylene dichloride-induced hypospadias was proposed: The ethylene dichloride metabolite, chloroacetaldehyde, increases expression of ATP3 which binds to CRE, preventing CREB from binding and decreasing transcription of genes containing the CRE element. Additionally, a computational chemical/protein docking model indicated interaction between CREB and 2-chloroacetic acid, another ethylene dichloride metabolite, which would also reduce CREB binding. NIH BLAST Genome Database results show that HSD3B2 promoter contains most of the CRE sequence, which supports the proposed association between the decreased transcription of gene HSD3B2 and hypospadias disease. These findings suggest that fetal ethylene dichloride exposure in utero may have contributed to the high incidence of hypospadias in Gela.
The CEBS database (https://manticore.niehs.nih.gov/celbsresearch/) catalogs toxicology study metadata and assay result information. Users can access the entire dataset, search, filter, download data, and verify results for unique test articles or compare the results for the same test article from different sources. In addition, these data are linked with the CEBS Search tool article reports so that users may easily navigate between the NTP Data Collection elements and other related data in CEBS. Together, the NTP Data Collection and CEBS Search functionalities expand understanding of the toxicological effects of test articles and facilitate easy access to all related data. The NTP Data Collection will be continuously updated with addition of new datasets in the future to support the needs of users.

The federal Tox21 consortium produces quantitative high-throughput screening (qHTS) data on thousands of chemicals across a wide range of assays covering critical biological targets. Many of these assays, and those used in other in vitro screening programs, rely on luciferase and fluorescence-based readouts which can be susceptible to signal interference by certain chemical structures resulting in false positive outcomes. Included in the Tox21 portfolio are assays specifically designed to measure interference in the form of luciferase inhibition and autofluorescence via multiple wavelengths (red, blue, and green) and under various conditions (cell-free and cell-based, two cell types). Out of 8,305 unique chemicals tested in the Tox21 interference assays, percent actives ranged from 0.5% (red autofluorescence) to 9.9% (luciferase inhibition) after filtering for curve class, efficacy, and cytotoxicity cutoffs. Bimodal potency distributions were observed among active chemicals, possibly due to differences in plasma protein binding. The penetration of 5-FU into human enterocytes after intravenous dosing (500 mg/m^2) for differences in plasma protein binding. The penetration of 5-FU into human enterocytes after intravenous dosing (500 mg/m^2) is well described, peaking 10 hrs after dose with an average number of apoptosis was well predicted in mouse - ratio between predicted and measured maximum on the first day and is recovered 16 days after chemotherapy. The agreement with histological data from biopsies that shows that apoptosis is decreased 3% by the end of the day and being recovered 16 days after dosing. This agrees with histological data from biopsies that shows that apoptosis is decreased 3% by the end of the day and being recovered 16 days after dosing. The simulation on a compute cluster can reduce the runtime by 99.7%, making it feasible for the software to analyze hundreds to thousands of expression datasets in less than a day, even on the whole transcriptome. Other notable improvements to the software and statistical methods include availability of annotations for GENIE extrapolated full transcriptome from the human platform, and new GCAT platforms (each with ~20K genes with linked GO terms); no (NOTEL) and lowest (LOTEL) observed transcriptomic adverse effect level calculations; Dunnett’s test for multiple comparisons; and non-parametric curve fits. These updates vastly improve the capabilities of the software and the ability to better model curves for chemicals eluding non-traditional dose responses. Important features include in vitro to in vivo extrapolation (IVIVE) method to broaden the scope and utility of the software. BMDExpress will continue to be maintained and developed to meet the expanding needs of toxicologists, risk assessors, and geneticists alike.

5-fluorouracil (5-fu) is a chemotherapeutic inducing intestinal apoptosis, an apoptotic marker in various toxicology, PBPK/PD positive enabling translation of preclinical predictions of adverse effects to human to guide drug development. In this work, a PBPK/PD model was built to describe 5-fu induced crypt cell apoptosis in small intestine of mouse and was extrapolated to human. The Simcyp Mouse Simulator v17 was used to model the PK of 5-fu with an oral dose of 260 mg/kg. Absorption followed first order dynamics and elimination was considered through liver, intestine and kidney. The gut tissue concentration was assumed to drive the cell damage model, included using a PD script. Stem cells and Transit Amplifying Daughter Cells (TADCs) divide in the intestinal crypts, TADCs differentiate into enterocytes, which migrate along the villi and die at its top for epithelium turnover. TADC apoptosis induced by 5-fu systemic concentration was well predicted in mouse - ratio between predicted and measured 1.12 for C_{max} and 0.52 for C_{TADC} and 1.37 for AUC. The evolution of apoptosis was well described, peaking 10 hrs after dose with an average number of apoptotic cells per crypt of 1.6, versus the observed 1.9 at 6 hrs. For human, the resulting exposure agrees with clinical data. The predicted average number of apoptotic cells per intestinal crypt reached a maximum of 0.5 at 5 hrs, having decreased 3% by the end of the day and being recovered 16 days after dosing. This agrees with histological data from biopsies that shows that apoptosis is maximum on the first day and is recovered 16 days after chemotherapy. The proposed PBPK/PD model is an initial attempt to describe the evolution of crypt cell apoptosis induced by 5-fu in mouse and human. This work contributes to the understanding, prevention and early detection of gastrointestinal toxicity. This work is supported by the TransQST grant.
unclear which factors have dominant influence on the assay result variation. In this study, meta-analysis was performed to identify possible cause of assay result inconsistency. Data for meta-analysis were collected from research articles that studied nanotoxicity on Daphnia magna according to the TGs. In data collection, physicochemical properties of NPs, experimental conditions of the assays, and the experimentally measured toxicities were extracted to compile the dataset. In total, 882 data points (NPs in each experimental conditions) were obtained from 83 publications. According to the analysis of the data, toxicity of non-coated NPs was not correlated with the TEM diameter of the NPs even though most of the NPs indicated higher toxic effects than non-NPs of identical core materials. To find out experimental conditions that caused assays result inconsistency, prediction models were developed for each type of NP (metal oxide, metal, and coated metal) with stepwise selection algorithm using support vector machine. Based on the model analysis, experimental treatments to disperse NPs in media were suggested as the most influential experimental conditions in varying assay results.

**1754 Transitioning to Composite Bacterial Mutagenicity Models in ICH M7 (Q)SAR Analyses**

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The International Council on Harmonisation (ICH) M7(R1) guideline describes the use of complementary (quantitative) structure-activity relationship (Q)SAR) models to assess the mutagenic potential of drug substance impurities in new and generic drug products. Historically, two statistical-based models have been used to predict mutations at G-C (guanine-cytosine) and A-T (adenine-thymine) sites, to comprehensively assess bacterial mutagenesis. In the present study, composite bacterial mutagenicity models using both G-C and A-T mutation types have been developed using two commercial statistical software platforms. These new models contain more than double the number of chemicals (n=9,067 and n=13,514) than the corresponding non-composite models with data harvested from the published literature, US FDA approval packages for drugs approved between 2009 and 2017, Centers for Food Safety and Applied Nutrition public databases, online repositories, and through data sharing efforts. Additionally, the use of composite bacterial mutagenicity models simplifies impurity analysis in an ICH M7 (Q)SAR workflow by reducing the number of model outputs requiring review. Cross-validation performance statistics for the new models range from 84 to 91% in sensitivity and 81 to 89% in negative predictivity. Additionally, an external validation set of 398 drug impurities representing proprietary pharmaceutical chemical space showed performance statistics ranging from 67 to 79% in sensitivity, 91 to 94% in negative predictivity and 94 to 96% in coverage. This data set was used in part to confirm that gaps in the applicability domain of the previous models were filled, while high predictive performance was maintained. This effort represents a major enhancement to (Q)SAR models that are recommended for use under ICH M7(R1), leading to improved patient safety through greater predictive accuracy, applicability, and efficiency when assessing the mutagenic potential of drug impurities.

**1755 The Impact of Chemical Quality on the High-Throughput Testing of Diverse Chemicals**

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Chemical quality is an important factor that can affect the bioassay results to cause false negatives/positives. The Tox21 10K chemical library has undergone analytical testing for quality control (QC) after being exposed to room temperature for 0 and 4 months (T1 and T2). Each chemical was assigned a QC grade based on purity, identity and concentration. In parallel, the Tox21 10K library has been tested in over 50 cell-based quantitative high-throughput screening (qHTS) assays. In this study, we used these data to analyze the correlation between chemical quality and bioassay activity as well as chemical structure. Chemicals with low QC grades at T2 were considered poor quality, and chemicals with a grade drop from T1 to T2 were considered unstable. Using the Random Forest (RF) method, we constructed and compared the performance of bioassay data and chemical structure-based prediction models. The prediction model was evaluated by the area under the receiver operating characteristic curve (AUC-ROC). The results show that poor quality chemicals were relatively less active than the chemicals that passed QC. Chemical quality and stability were found to correlate with chemical structure significantly with stability showing a better correlation than quality. The AUC-ROC of the best model was as high as 0.75. This indicates that chemical structure is a good predictor of chemical stability. The models also identified structure features of unstable chemicals, such as Nedaplatin, Endrin, Glycobiocin. In addition, the Tox21 p53 assay was used to test the 10K library at T1 and T2. About 2% of the library showed a significant change in activity between T1 and T2 turning from either active to inactive or inactive to active. The correlation between this activity change and chemical stability was analyzed. Taken together, these results could serve as a guidance for interpreting the Tox21 assay results and for future chemical selection and handling.

**1756 Bioinformatic Integration of In Vivo Data and Literature-Based Gene Associations for Prioritization of Adverse Outcome Pathway Development**

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Adverse outcome pathways (AOPs) describe a sequence of events, beginning with a molecular initiating event (MIE), proceeding via key events (KEs), and culminating in an adverse outcome (AO). A challenge for use of AOPs in a safety evaluation context has been identification of MIES and KEs relevant for AOs observed in regulatory toxicity studies. In this work, we implemented a bioinformatic approach that leverages mechanistic information in the literature and the AOs measured in regulatory toxicity studies to prioritize putative MIES and/or early KEs for AOP development relevant to chemical safety evaluation. The US Environmental Protection Agency Tox21 Reference Database (ToxRefDB) contains chemical and biological information for curated chemicals ranging from >5000 studies or summaries from sources including data evaluation records from the US EPA Office of Pesticide Programs, the National Toxicology Program (NTP), peer-reviewed literature, and pharmaceutical preclinical studies. To increase ToxRefDB interoperability, endpoint and effect information was crosswalked into SMIRX from the United Medical Language System, which enabled mapping of in vivo pathological effects from ToxRefDB to PubMed (via Medical Subject Headings or MeSH). This enabled linkage to any resource that is also connected to PubMed or indexed with MeSH. A publicly available bioinformatic tool, the Entity-MeSH Co-occurrence Network (EMCON), uses multi-source data sources and a measure of mutual information to identify genes most related to a MeSH term. Using EMCON, gene sets were generated for endpoints of toxicological relevance in ToxRefDB linking putative KEs and/or MIES. The Comparative Toxigenomics Database was used to further filter important associations. As a proof of concept, thyroid-related effects and their highly associated genes were examined, and demonstrated relevant MIES and early KEs for AOPs to describe thyroid-related AOs. The ToxRefDB to gene mapping for thyroid resulted in >50 unique gene to chemical relationships. Integrated use of EMCON and ToxRefDB data provides a basis for rapid and robust putative AOP development, as well as a novel means to generate mechanistic hypotheses for specific chemicals. This abstract does not necessarily reflect US EPA policy.

**1757 A Workflow to Assist in Expert Review and Regulatory Submissions of ICH M7 (Q)SAR Assessment of Impurities**

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After the implementation of the International Conference on Harmonisation’s (ICH) M7 guideline, (Q)SAR approach has become particularly important in assessing bacterial mutagenicity potential of drugs and impurities. The guidance recommends including outcomes from both statistical and expert rule-based models and to perform expert review of the raw output of the (Q)SAR predictions with the help of supporting information. Application of expert knowledge improves accuracy, increases confidence, and provides a rationale for the assessments. However, steps required for such review and documentation are still challenging. In this study, we report a workflow designed to combine the results of highly predictive statistical and expert rule-based models and to generate a variety of supporting evidence with the goal to assist expert review and regulatory submissions. Supporting evidence includes structural relationships between the active drug substance and the impurity, analysis of structurally similar analogs, evaluation of the relevance of identified alerts and analysis of the structural features not covered by the (Q)SAR models. The workflow suggests an overall call and ICH M7 class for bacterial mutagenicity assessment and provides a confidence level as well as useful tips for human expert review, highlighting the areas with strong and weak evidential support. Special care is taken to resolve out of domain and uncertain outcomes. To validate the workflow, a test of 407 chemicals (155 positives/252 negatives, data from proprietary contributors) was performed against bacterial mutagenicity statistical and expert rule-based models. After applying the workflow, the most notable effects were in the increase of true positives by 20% and 6% increase in the structural coverage. Overall 72% sen-
Comparative Case Studies to Establish a Standardized Process for Read-Across within a Daily Safety Assessment Workflow

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Read-across is one of the alternative methods considered for regulatory purposes to fill data gaps encountered in product safety assessments. The toxicity potential of a chemical is inferred from known toxicity of compound(s) having a similar structure and property profile. Nevertheless, read-across is an evolving method with several open issues, one of which is related to the lack of consensus regarding the extent and type of evidence necessary to support a read-across. To achieve reliable read-across results, a quantitative and transparent methodology is required for similarity assessment as well as hypothesis-driven evaluation. We applied commercial and public software tools to establish standardized workflow. Chemical safety of two cosmetic ingredients (Isopropyl palmitate and Neopentyl Glycol Dicaprate) were assessed by analogue-based read-across method for target evaluations including Point of Departure (POD) estimation and potential value content/developmental safety, sensitization, and genetic toxicity. The chemical similarity was compared for structural fingerprints from multiple sources (RDKit, MCCS Keys, ToxPrints, and CDK) using ChemTunes, ToxGPS® and AMBIT. Properties-based similarities were calculated from ToxGPS. Toxicity data were compiled from ChemTunes and AMBIT databases. TIMES-SS and ChemTunes LiverBioPath was used to address metabolites and reaction similarity. Final combinations of diverse evidence based on different similarities were performed using the quantitative weight-of-evidence approach available within ToxGPS Read-Across tool. Metabolic similarity gave rational hypothesis along with read-across scenario which is region of select appropriate analogues. Isopropyl palmitate as isopropyl palmitate as well as Neopentyl glycol dicaprylate, Pentenythryltetraesterate and Trimethylolpropane trinonanoate for Neopentyl Glycol Dicaprate were selected as analogue with regard to metabolic similarity. This case study demonstrated identification of reasonable analogues by the complementary use of commercial and public read-across tools. Systematic and reproducible outcomes can be attained along with estimation of the associated uncertainty. This standardized workflow can be applied for day-to-day safety assessment.

BioCelerate Sendharmonization Initiative: A Proposal to Better Harvest the Value from Send Data

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BioCelerate, a subsidiary of TransCelerate BioPharma, Inc., is a preclinical industry consortium driving initiatives to increase efficiency and productivity in early stage R&D. The Standard for Exchange of Nonclinical Data, or SEND, identifies an approach to gathering and representing nonclinical data in a standardized workflow workflow. Safety, efficacy, and regulatory submissions. The consortium is currently engaging stakeholders to clarify the problem statement and to begin to understand options for future harmonization of SEND through CDISC. The implementation of these recommendations is expected to improve the relevance of these studies from this initiative will allow comparison of warehoused SEND data and unlock value that is currently unrealized. These comparisons include the ability to compare the progression of findings over time with a single molecule and a comparison of target organs across multiple molecules directed against the same target. This poster/presentation outlines the breakdown of the methodology for mapping of SEND data variability and the cross-stakeholder engagement framework proposed to identify solutions for SEND data harmonization, ultimately contributing to the goals of increased efficiency and productivity in early stage R&D.

Development of an Adverse Outcome Pathway (AOP) Network for Carcinogenicity Using Expert-Derived (Q)SAR Knowledge


The prediction of carcinogenicity and related toxicity endpoints has always been a principal area of research for in silico (Q)SAR systems and as a result, software relating to these endpoints is well developed. Indeed, in recent years predictions provided by these systems have become embedded in regulatory guidance, where they may be used to replace or augment other testing methods. Consequently, it is important that the predictions are as accurate as possible and are provided in such a way that they can be easily integrated with other sources of evidence. Derek Nexus (DX) is an expert rule-based SAR system with a well-developed knowledge base for carcinogenicity and related endpoints. Within this knowledge base, there is information on molecular-initiating events (MIEs), as well as other potential key events (KEs) and modes of action (MoA) associated with the compound classes covered by the alerts. In this work, we investigated 310 alerts related to carcinogenicity in DX and used the knowledge contained in the alerts. These linkages provided a skeleton AOP network containing 26 AOPs. This network was then subjected to a detailed review using public literature to supplement the AOPs with evidence for biological plausibility, and information such as species relevance. This review dramatically increased the scope of the network, with the number of pathways associated with the 26 MIES increasing from around one pathway per MIE to more than twice as many. The review also allowed for the association of each pathway with events at the protein level. This network can be used as a rudimentary profiling tool for carcinogenicity MoA prediction. A combination of literature review and profiling of carcinogenicity AOPs using our model also identified additional MIEs and KEs for future investigation and integration into the network. It is hoped that this approach to knowledge presentation will allow for easier interpretation of the evidence available relating to a given prediction. Presenting more detailed information on potential pathways allows for better integration of existing and emerging in vitro and in vivo tests at the protein level with predictions produced by DX. This methodology also allows for expansion of the scope of predictions which can be made for this endpoint, allowing for integration of MIES, ADME data and other data not necessarily related to toxicity outcomes.

Machine Learning Approaches to Categorize Carbonaceous Nanomaterials Based on Patterns of Inflammatory Markers and Pathological Outcomes in Lungs

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As technology advances to incorporate nanoparticles (NP) in various industries, the exposures associated with these particles also increase. Exposure to carbonaceous NPs has been found to be associated with substantial pulmonary toxicity, including inflammation, fibrosis, and/or granuloma formation. Despite several attempts made previously, grouping or categorizing NPs based on their intrinsic properties and certain inflammatory endpoints remains a challenge. The inconsistency and a large number of variables across studies considered by different groups for evaluating toxicity responses of NPs, the lack of precise understanding of the role of different NP characteristics on various biological responses, as well as missing NP data under in vivo biological conditions and pathological outcomes often the result of chronic inflammatory responses further complicates the selection of NPs. This study attempts to categorize the toxicity profiles of various carbon allotropes, in particular, carbon black, different multi-walled carbon nanotubes, graphene-based materials and their derivatives. Statistical and machine learning based approaches were used to identify groups of CNMs with similar pulmonary toxicity responses from a panel of proteins measured in bronchoalveolar lavage (BAL) fluid samples and with similar pathological outcomes in the lungs. Thus, grouped particles based on their pulmonary toxicity profiles, were used to select a small set of proteins that could potentially identify and discriminate between the biological responses associated within each group. Specifically, MDC/CCL22 and MIP-3β/CCL19 were identified as common protein markers associated with both toxicologically distinct groups of CNMs. In addition, the persistent expression of other selected protein markers in BAL fluid from each group suggested their ability to predict toxicity in the lungs, i.e., fibrosis.

Pathway (AOP) Network for Carcinogenicity Using Expert-Derived (Q)SAR Knowledge

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1762 Identifying the Link between Chemical Exposures and Incidence of Triple-Negative Breast Cancer in African American Women

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The incidence of triple negative breast cancer (TNBC), an aggressive subtype of breast cancer for which there is no targeted therapy, is approximately three times higher in non-Hispanic black (NHb) women compared to non-Hispanic white (NHW) women. The mechanisms driving this difference are unknown, and likely lie in an interaction between genetic and environmental factors. Here, we aimed to identify chemical exposures which may play a role in TNBC disparities. Using chemical biomonitoring data from the National Health and Nutrition Examination Survey (NHANES) and biological activity data from the US EPA’s ToxCast program, we identify chemicals at higher concentrations in NHb women and assess their toxicological relevance to breast cancer risk. A total of 44 chemicals showed significantly higher biomarker concentrations in NHb women. Investigation of these chemicals in ToxCast resulted in a total of 22,061 assays for analysis, 5,343 of which contains adequate modl_ga (logAC50) and modl_tp (scaled top value of dose response curve) data. BPA, known to be associated with breast cancer, and PFOS were most tested, and had 19.98% and 20.46%, respectively, of assays tested reported as active. Of interest are PFDA, PFUnDA, PFNA, and Chlordane due to their higher concentrations in NHb women and moderate testing and activity in ToxCast. Furthering our chemical prioritization, Gene Set Enrichment Analysis (GSEA) provided pathway association between active genes analyzed in ToxCast and putative TNBC mechanisms. Using publicly accessible high throughput data, we have prioritized chemicals of interest to further investigate for their relevance in TNBC disparities.

1763 Integrate Mechanistic Knowledge with High-Throughput Data to Assess Risk for Drug-Induced Liver Injury Using Adverse Outcome Pathway Networks


Due to the well-known limitations for current animal testing based approaches to predict drug induced liver injury (DILI), there is heightened interest in incorporating high throughput assays into the evaluation framework for DILI risk. However, the diverse and high dimensional nature of these data poses serious challenges for common data mining and machine learning techniques even with recent advances. Integrating high throughput assay information with mechanistic knowledge in the form of expert opinion and literature findings might provide a promising approach to fully utilize the power of both mechanistic understanding and new testing technologies. In this presentation, we discuss our pilot study using adverse outcome pathway (AOP) networks to provide a base model for incorporating high throughput data from L1000, CMap, and Tox21 for gene expression changes and nuclear receptor binding. AOP networks were formed by integrating published AOPs for liver steatosis, cholestasis, fibrosis, and liver tumor. Information for relevant nuclear receptors and genes was then extracted from these networks. We obtained measurements on nuclear receptor binding and differential gene expression for a collection of drugs in the Liver Toxicology Knowledge Base. A rule ensemble learning model was then built to infer liver toxicity from these molecular predictors resulting in competitive performance with other approaches. Our result suggests that current knowledge encoded in AOPs can be successfully utilized for dimension reduction for high throughput data and leading to capable predictive models. With continued improvement in AOP development and new testing technologies, combining mechanistic insight with high throughput data holds great promise in advancing DILI risk assessment.

1764 QSAR Models for Mean LD50s Are More Predictive Than for Minimum LD50s

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In April 2018, ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods) held a workshop on "Predictive Models for Acute Oral Systemic Toxicity." The Committee invited participants to develop in silico models to predict median lethal doses (LD50s) for a large dataset of chemicals they had curated. A subset of chemicals were included with multiple LD50s per chemical. For this subset, the Committee selected the more protective LD50 point estimate. Based on LD50 variability (Siwakoti et al., 2018), a minimum LD50 will necessarily carry greater experimental uncertainty. We hypothesized that using the mean LD50 instead of the minimum would result in QSAR models with better predictive ability. To test this idea, identical model-building conditions were used to develop models for both the mean LD50 and the minimum LD50 as response variables. The overall predictive ability of the global models is moderate, but the mean LD50 was a consistently better response variable than the minimum LD50. For incorporation of alternative models in risk assessment, this exercise demonstrates the value of characterizing the variability in the endpoint to be modeled. Disclaimer: the findings and conclusions in this presentation have not been formally disseminated at 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.

1765 Reconciling In Vitro and In Silico Approaches for Drug-Induced Liver Injury Prediction


Drug-induced liver injury (DILI) is one of the main reasons for drug attrition during clinical trials and of withdrawal from the market. This makes the early identification of hepatotoxicity of compounds a critical challenge. In silico hepatotoxicity prediction models make a cost-effective approach able to prioritize compounds for preclinical and clinical studies. Recent efforts have been made to create more accurate quantitative structure-property relationships (QSPR) models relating hepatotoxicity to chemical structure features. However, to this date only few have integrated in vitro data to their models. This study aims at integrating in vitro activation signal of stress response pathways involved in DILI to standard molecular description of compounds to better identify substructures inducing hepatotoxicity. A library of 118 compounds was screened on a panel of 8 previously established HepG2 BAC-GFP reporter cell lines that capture endoplasmic reticulum (ER) stress, DNA damage, heat shock response and inflammatory responses. Fluorescence microscopy images were taken at 24, 48 and 72 hours after addition of the compounds (concentration ranging from 1 to 100 Cmax). Additionally, propidium iodide (PI) and annexin V (AnxV) staining was performed to detect necrotic and apoptotic cells. Quantitative image analysis was performed with CellProfiler. GFP integrated signals, Cmax, AnxV and PI values were used as descriptors of the compounds along with extended connectivity fingerprints of radius 3 (ECFP, 6) physicochemical descriptors of the compound structures. Statistical models (Bayesian, Random Forests, Gradient Boosted Decision Trees) were applied to relate both in vitro derived data and molecular structure description to the US FDA approved DILI annotation (Most, Less, No and Ambiguous DILI concern). Our results demonstrate that the integration of both in silico chemical descriptors and in vitro quantitative mode-of-action data increased the predictive performance of the predictive models. This work highlights the importance of integrating both in silico and in vitro approaches in the construction of predictive models for DILI. This work was supported by the NIMH/ETRANSAFE project (grant agreement 777365) and the H2020 EU-ToxRisk project (grant agreement 681002).

1766 Database of Pharmacokinetic Time-Series Data and Parameters for Environmental Chemicals

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Time courses of compound concentrations in plasma are used in chemical safety analysis to evaluate the relationship between external administered doses and internal tissue exposures. This type of data is experimentally generated for chemicals like pharmaceuticals or cosmetics, but is not usually available for the thousands of other chemicals to which people may potentially be exposed. An understanding of the pharmacokinetics for these chemicals can be important for modeling in vitro assays and in silico models, but the certainty of the quantitative application of these estimates to chemical safety evaluations
cannot be determined without in vivo data for external validation. To address this need, we present a public database of time-series from almost 600 studies in humans or test animals for over 150 environmentally-relevant chemicals and their metabolites by all major administration routes with concentrations measured in blood/plasma, tissues, and excreta. All information was sourced from either open databases or published literature sources identified using machine learning. The extracted pharmacokinetic time-series are stored in a MySQL data model with detailed, manually curated metadata. Concentration-time points were manually captured using the webtool WebPlotDigitizer. Data in all fields are normalized to facilitate comparisons, but original values are retained for transparency. Plots of the data will be viewable on the US EPA ComToxChem Dashboard (https://comptox.epa.gov/dashboard) along with optimized pharmacokinetic summary parameters (e.g. area under the curve, volume of distribution) calculated using the R package invivoPKfit.

In addition to model calibration and validation, this set may be used for analyses of differential chemical distribution across chemicals, species, doses, or routes, and can be added on pharmacokinetic studies performed in vivo can also host data and metadata needed for metabolism and pharmacokinetics testing guidelines. To facilitate data sharing we can accommodate (and encourage) submissions of structured published data on any compound. This abstract does not necessarily reflect US EPA policy.

1767 Application of HTTK Pregnancy Modeling and IVIVE Approaches for High-Throughput Screening


The US EPA has developed a high throughput toxicokinetic (HTTK) modeling platform to support animal-free toxicity predictions using in vitro to in vivo extrapolation (IVIVE). Recently, HTTK has been extended to describe pregnancy (fetal HTTK). To test the performance of the fetal HTTK platform in modeling developmental toxicity from in vitro findings, we sought to compare an exposure-based prediction of developmental toxicity with human induced pluripotent stem cell responses to 7 retinoid analogs recently reported by Palmer et al. (2017): all-trans-retinoid acid (ATRA), Retinol, Acitretin, Eretinate, 9-cis-retinoid acid (9-cis-RA), 13-cis-retinoid acid (13-cis-RA) and 4-(E)-2-(5,6,7-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl benzoic acid (TTNPB). The fetal HTTK model describes human pregnancy starting from the end of first trimester and spanning second and third trimesters. We assumed that the external dose predicted by reverse dosimetry in the non-pregnancy condition reflects early stages of pregnancy, and then used the fetal HTTK model simulations to predict pregnancy-induced changes in internal dosimetry. Based on the predicted external dose to result in an internal exposure level of 0.01 μM (i.e., the teratogenic index predicted by the stem cell assay for ATRA), the rank order of retinoids was ATRA>Retinol>Acitretin>Eretinate>9-cis-RA = 13-cis-RA > TTNPB. This rank order was based on predicted external dose followed by that of the stem cell response. Assuming the pharmacokinetic disposition of these analogs reach steady state prior to pregnancy, the fetal HTTK model predicted an overall decrease in maternal plasma concentrations over the course of gestation with a maximum decline of 8.5% in second trimester (for Eretinate) and 14.9% in third trimester (for TTNPB). The model also allowed for the prediction of concomitant fetal exposure which showed an overall decreasing trend as well (about 14.7%). Furthermore, we predicted the analog-specific external exposure doses as determined in the stem cell assay to be 2.2e-03, 1.1e-05, 6.2e-06, 4.9e-04, 6.0e-01, 8.2e-07 mg/kg/day for ATRA, 13-cis-RA, 9-cis-RA, Eretinate, Retinol, TTNPB, respectively. These results demonstrate the utility of predictive modeling coupled with animal-free in vitro experimentation to evaluate developmental toxicity potential in support of high throughput screening of chemicals. This abstract does not reflect US EPA policy.

1768 Computational Model for Inhibition of Mitochondrial Function

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In eukaryotes, mitochondria play many life-sustaining roles, with their dysfunction linked to toxicity as well as numerous diseases. We present the development of a computational model that predicts whether a xenobiotic will or will not inhibit mitochondria, with some sub-target information (e.g., inhibition of Complexes I-V or uncoupling via protonophore action). We compiled public databases that target the mitochondria together with associated control compounds that do not alter mitochondrial function. We employed molecular scaffolding and fingerprinting to identify conserved structural motifs and features associated with mitochondrial inhibitors versus controls. By using these attributes as covariates within random-forest machine-learning models, we can identify mitochondrial inhibitors with high sensitivity (83%), specificity (79.3%) and accuracy (81%). The high negative predictive value (93.7%) of our model highlights confidence with which novel compounds can be flagged as mitochondrial agents. When tested against a database of therapeutic candidates withdrawn from the market due to liver injury, our model rapidly identified those that had subsequently been shown to target mitochondria. Furthermore, we were able to identify two compounds that were recently identified as mitochondrial toxins only via experimental testing, highlighting the utility of our model. Given its speed and ease of use, our approach can be used as an integrated approach that complements in vitro mechanistic screening and as a covariate in models that address the etiology of complex biological endpoints. We will make our model publicly available to allow rapid assessment of compounds under development.

1769 Probabilistic Prediction of Human Skin Sensitiser Potency for Use in Next Generation Risk Assessment


Our aim is to develop and apply next generation approaches to skin allergy risk assessment that do not require new animal test data address novel exposure scenarios and better quantify uncertainty. We have developed a Bayesian multi-level regression model to estimate the human sensitiser population threshold (defined as, the chemical-specific exposure level at which no individual in a population will experience induction of contact allergy) under the conditions of a human repeat insult patch test (HRRIPT). Our approach is built upon dose response modelling of historical human data and allows low predictions of human sensitiser potency to be made using historical murine local lymph node assay (LLNA, OECD TG 429) data and/or in vitro test method data (DPRA (OECD TG 442C), KeratinoSensSM (OECD TG 442D), h-CLAT (OECD TG 442E) and U-SensTM (OECD TG 442E)). A key feature of the approach is that the uncertainty in any prediction is explicitly quantified. Our Bayesian probabilistic model is used to estimate population thresholds for 30 chemicals using a weight of evidence incorporating previously published HRRIPT, LLNA, DPRA, KeratinoSensSM, h-CLAT and U-SensTM data. Estimates for a further 43 chemicals using in vitro test method data only are also presented. Comparisons are made with current risk assessment metrics and across data types. This analysis suggests that estimates of human potency generated from in vitro data alone have at least the same level of accuracy, on average, as estimates generated from LLNA data. Consequently, we propose that this approach can be used to derive a point of departure for next generation risk assessment and have submitted it for consideration by the OECD Defined Approach Skin Sensitisation (DASS) Expert Group as ‘Skin Allergy Risk Assessment Defined Approach’ or SARA DA. Application of the SARA DA to four theoretical skin allergy risk assessment case studies (caffeine, coumarin, curcumin and sulforaphane) will be presented, each addressing a different product exposure scenario, to illustrate how the DA prediction can be used as part of a weight of evidence decision-making approach.

1770 Evidence Integration across Multiple Toxicity Endpoints to Prioritize Constituents of Potential Concern in Oil and Gas Produced Water

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In the United States, onshore oil and gas extraction operations generate an estimated 900 billion gallons of produced water annually making it the largest waste stream associated with upstream development of petroleum hydrocarbons. Management and disposal practices of produced water vary from injection into underground storage wells to “beneficial reuse” of produced water in agricultural settings. Recent efforts, including development of the FracFocus database, have provided insights into some of the chemical constituents used in the hydraulic fracturing extraction process. However, there is relatively little information with regard to the potential for the chemical compounds in produced water. Therefore, this project aimed to address the gaps in our understanding of potential risks to human health or environmental impacts from various disposal practices. We performed a comprehensive literature review, screening nearly 16,000 published articles, to identify chem-
icals that have been detected in produced water. Searches for information on
the potential ecological or mammalian toxicity of these chemicals revealed
that the overwhelming majority of these substances have not been a subject
of safety evaluation or mechanistic toxicology studies. In order to integrate
available evidence and fill in data gaps, we have catalogued available data
from ecotoxicity studies and toxicity screening databases and predicted
toxicity values using Toxicological Priority Index (ToxPI) approach. This
research effort will inform stakeholders and decision-makers on the potential
hazards and risks of this complex wastewater. In addition, this project created
a prioritized list of compounds that, based on hazard and potential exposure,
may be included in the monitoring strategies to reduce those possible human
health risks and environmental impacts.

1773 In Silico Modelization of Compounds Interaction with Bile Salt Export Pump (BSEP): An Alternative Approach to Predict Hepatotoxicity

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BSEP is an efflux transporter protein present in the hepatocytes membrane that plays important role in flow of bile acid from hepatocyte cell into the bile canaliculi (1,2). Impaired BSEP activity due to drug interaction leads to accumulation of bile acid within the hepatocyte and results in cholestasis and liver injury (DILI)(3). However, there is no detail information about interaction of compounds with BSEP. To develop 3D model for BSEP transporter protein and analyzing its affinity with compounds using docking tool, the 3D model of BSEP (Uniprot code: Q9YQ30) was determined by homology modeling, and further validated. US FDA-approved compounds were selected and docked against the BSEP structure. The post docking complex was analyzed in order to evaluate the behavior of compounds with BSEP. Homology modeling produced 3D model of BSEP using mouse p-glycoprotein as template with more than 70% homology. Ramachandran plot confirms that 3D model has more than 90% of amino acid residues in the most favored regions. Molecular docking showed that bosentan (positive control) produced higher affinity for BSEP as compare to the caffeine with docking energy of -15.21 kcal/mole (IC50 = 38.1 μM for bosentan) - 4.60 kcal/mole (IC50 > 135 μM for caffeine). Our in silico approach confirmed that bosentan has very high affinity compared with caffeine and acts as an inhibitor of BSEP which plays major role in formation of cholestasis. Therefore, our in silico approach may be helpful to design in vitro experiments using BSEP in order to predict precisely the role of compounds in hepatotoxicity. 1: Kis E, Toxicol in vitro. 2012 Dec;26(8):1294-9, 2: Yang K, J Pharm Sci, 2013 Sep;102(9):3037-3037. 3: https://www.solvobiotek.com/trans-

1774 Optimum Concentration-Response-Curve Metrics for Toxicity Prediction Based on High-Content Cellular Imaging


High-content imaging (HCI) enables rapid in vitro assessments of complex cellular phenotypic changes (e.g. cell morphology or protein localization) induced by chemicals. Supervised classifiers may be constructed based on the phenotypic readouts of reference chemicals, and used to predict the toxicity of new chemicals to specific human tissues or organs. Changes in readouts are often measured at multiple concentrations and modeled using concentration response curves (CRCs), and metrics derived from CRCs may be used as classifier inputs. Commonly used metrics include potency measures, such as half-maximal effective concentration (EC50), and efficacy measures, such as maximal response (Rmax). However, it is unknown which metrics for in vitro readouts yield the most accurate classifiers for in vivo toxicity. We studied 16 different metrics: nine potency metrics, effective concentrations at 10 to 90% activity (EC10, EC20, EC30, EC40, EC50, EC60, EC70, EC80, EC90), and seven efficacy metrics, responses at 31 to 2000 μM (R31, R61, R100, R300, R600, R1000). Data came from two previous studies of 42 or 33 chemicals and 129 or 166 readouts in human kidney (HPTC and HK-2) or lung (BEAS-2B and A549) cells, respectively. The chemicals were annotated according to their known in vivo toxic effects to the specific cell types, and the cells were exposed to chemicals for 16 hours at seven distinct concentrations. For each readout, we trained linear kernel support vector machines to classify the chemicals into “toxic” and “non-toxic” groups. For each dataset, around 2064 to 2656 classifiers were trained, with balanced accuracies assessed using 10-fold cross-validation. We found that efficacy metrics gave greater proportions of “top-performing” classifiers (those with the top 10% accuracy over all the trained classifiers) than potency metrics for all data-

1772 Determination of Plasma Protein Binding via Ultracentrifugation and In Silico Modeling: Can We Trust the Results?

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In the context of broader application of alternative toxicological testing and thereof derived risk assessments, in vitro obtained effect concentrations need to be related to external, e.g. oral doses (in vitro - in vivo extrapolation (IVIVE)). Toxicokinetic modeling, a key element in IVIVE, can be realized by the application of e.g. PBTK models. One of the relevant input parameters for PBTK modeling is the plasma protein binding (PPB) that is routinely determined by rapid equilibrium dialysis (RED). Here, we present PPB determinations by in silico and experimental determination of RED. Ultracentrifugation was performed for rat plasma samples spiked with 5 μM of each test compound. Samples were centrifuged for 18 hours, at 37°C, 215,000 x g and the supernatant was analytically measured by LC-MS/MS to estimate the fraction unbound (Fu). Modeled data were generated by a QSAR model developed to predict PPB. Fu in UC samples could be successfully determined and, except for FLU, yielded data of 86 % (APAP), 74 % (CAF) and 77 % (COL) coincide with Fv to the literature (82, 84, and 76 % Fv for APAP, CAF, COL and RED data (79, 65 and 76 %). Modeled data for CAF (68 %) and COL (60 %) fit well in the experimental and literature data, whereas modeled Fv of APAP (92 %) shows a better accordance to UC data (86 %) in comparison to RED results and the literature. Fv of FLU (26 %) determined by UC does not coincide to literature and RED data with 4.7 and 5.7 %. In addition, modeled data predicts a Fv of 5-10 % for FLU which is comparable with RED and literature data. Physicochemical properties could play a role in PPB as FLU is a more lipophilic substance in comparison to the other test compounds (log P<sub>O/W</sub> 3.35). In conclusion, our work demonstrates alternative methods for PPB affinities that should be applied to more substances, e.g. lipophilic or instable substances for RED experiments. We recommend the expansion of data to get a better knowledge on PPB depending on the physicochemical properties of different chemicals and its influence on toxicokinetic modeling.

1771 Exploring Current Read-Across Applications and Needs among US Federal Agencies


United States Federal agencies are tasked with protecting human health and the environment by determining the potential health hazards posed by chemical exposures and consumer products. Testing all such substances using traditional animal-based methods poses time, cost, and practical and ethical challenges. With this in mind, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) has released a strategic roadmap for establishing new approaches and methodologies to evaluate the safety of chemicals. Read-across is one such alternative method for filling data gaps in the hazard profile of a substance of interest using existing data from chemical analogs. In addition to providing information quickly and without the use of additional animal experiments, read-across can be tailored to be related to external, e.g. oral doses (in vitro - in vivo extrapolation (IVIVE)). Modeled data were generated by a QSAR model developed to predict PPB. Fu in UC samples could be successfully determined and, except for FLU, yielded data of 86 % (APAP), 74 % (CAF) and 77 % (COL) coincide with Fv to the literature (82, 84, and 76 % Fv for APAP, CAF, COL and RED data (79, 65 and 76 %). Modeled data for CAF (68 %) and COL (60 %) fit well in the experimental and literature data, whereas modeled Fv of APAP (92 %) shows a better accordance to UC data (86 %) in comparison to RED results and the literature. Fv of FLU (26 %) determined by UC does not coincide to literature and RED data with 4.7 and 5.7 %. In addition, modeled data predicts a Fv of 5-10 % for FLU which is comparable with RED and literature data. Physicochemical properties could play a role in PPB as FLU is a more lipophilic substance in comparison to the other test compounds (log P<sub>O/W</sub> 3.35). In conclusion, our work demonstrates alternative methods for PPB affinities that should be applied to more substances, e.g. lipophilic or instable substances for RED experiments. We recommend the expansion of data to get a better knowledge on PPB depending on the physicochemical properties of different chemicals and its influence on toxicokinetic modeling.
1775 A Unified Approach for the Analysis of Zebrafish Developmental and Neurotoxicity Data: A Multi-Lab Case Study
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Zebrafish are gaining acceptance as a screening tool for developmental toxicity (DT) and neurotoxicity (NT). Currently, there is no formal guidance on how to most effectively perform and analyze the results from these studies. To start the process of formalizing such testing standards, the NTP has formulated a library of 87 compounds (including pesticides, flame retardants, drugs, industrials, polycyclic aromatic hydrocarbons) with diverse toxicological properties. These 87 compounds were evaluated for DT and NT effects in zebrafish in three labs. The protocol designs varied by: embryos with/without chorions, time point of exposure and response measurement, and tested concentrations. To develop a unified data analysis approach across all data sets, we harmonized the data, and adopted a benchmark concentration (BMC) approach to estimate potency of the effect. Our BMC approach used a benchmark response (BMR) based on the intrinsic response variation of each endpoint. Bootstrap statistics were used to generate a confidence interval around the BMR. This standard approach allowed us to compare the data across labs, and across three labs. Comparisons were based on various parameters such as BMR, response variation (e.g., standard deviation, SD) in vehicle control (i.e., DMSO), and embryo behavior response similarity in vehicle control in the NT assay, etc. The concordance of the active chemicals identified by the BMC approach and benchmark was high in the DT assay than in the NT assay. Specifically, the active chemical concordance between labs in the DT (embryotoxicity included) assay was 73%, 89%, 70%, and across labs was 65%. For NT assay, the active chemical concordance between labs was 62%, 71%, 67%, and across labs was 44%. The pooled SD of BMC values from the concordant active chemicals in DT and NT assay was 0.47 and 0.52 (in log10 unit), respectively, which was about the spacing between two consecutive concentrations tested, approximating the sensitivity of the protocol design. Pesticides and flame retardants were the two most prominent chemical classes in the concordant active chemicals, covering 57% (17/30) in DT assay and 80% (12/15) in NT assay, respectively. This is the first time that such a unified data analysis approach has been implemented for zebrafish toxicity screening data and offers a novel tool to begin to harmonize testing across labs and protocols.

1776 Development of an RNA-Seq Tissue Expression Atlas of Preclinical Species (Cynomolgus Monkeys and Beagle Dogs) for In Silico Assessment of Target Expression and Distribution

A better understanding of drug targets and their tissue expression patterns in preclinical species is critical for demonstrating relevance to humans and supporting species selection for First-In-Human (FIH)-enabling nonclinical safety studies predicated on developmental and reproductive toxicity. This significance has been highlighted by the recent release of the revised European Medicines Agency Guideline for FIH and early clinical trials. The development of RNA-Seq technologies has improved our ability to generate quality target gene expression data and has been successfully applied to characterize human gene expression patterns in preclinical species. A standardized approach allowed us to compare the data across labs, and across nine labs. The goal of this project was to use RNA-Seq to profile a broad collection of tissues from a number of commonly used nonclinical species to build a comprehensive tissue expression atlas complementary to human tissue transcriptomic maps (e.g., GTex) for rapid assessment of target distribution. Frozen tissues from cynomolgus monkeys and beagle dogs were collected based on coverage with early toxicity and GLP study designs and totaled to over 60 tissues per species. Samples were dissected and harvested from 3 male and 3 female animals with minimal euthanasia time. Sampling and subsequent sorting of blood cells from a set of 3 male and 3 female animals was accomplished separately. Following collection, RNA isolation, library preparation, sequencing, alignment and feature counts were performed to produce measurements of transcript abundance. Tissues from the same organ systems clustered together and overall human to nonclinical species correlation was above 60% (R-squared). These datasets can also complement other information such as functional assay data to support the selection of pharmacologically relevant species for safety evaluation and de-risking studies of small molecule and biotherapeutics. Additional tissue maps are also being generated for rat and monkey to create a complete and authoritative preclinical transcriptomic tissue map resource.

1777 A Large Two Stage Multi-Task Network for Chemical Properties
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Neural networks enable transfer learning where models trained on one task accelerate learning on other tasks. We show transfer learning models result in accuracy improvements across a variety of chemical endpoints. The UL Cheminformatics Tool kit models 74 different chemical endpoints including acute oral-, dermal- and inhalation-toxicity, dermal sensitisation, dermal- and eye-irritation, genotoxicity, and acute- and chronic-aquatic toxicity. These models are constructed in two stages. Stage (1) constructs a neural network for every endpoint from chemical structural data. Stage (2) constructs networks with inputs taken from Stage (1) hidden layers. Data Isolation in Conversions (DIC) models are created to link traditional toxicological data to computational modeling. These models first collect chemical property data, then collect chemical structural data, and finally build a supervised learning model that inputs chemical structural data and outputs predicted endpoint data. This type of model fails to leverage data from disparate sources. Even with these limitations, the Stage (1) models achieve high accuracies of 70-80% across the nine models. Multi-task Learning (MVC) replaces the Stage (2) models with isolated data by linking useful representations between models trained on different data sets. The two stage model benefits by easing the integration of new data. Model training is not constrained to a single set of data but can make use of all available data. These multi-task models can only result in changes in the embedded layers shared to the second stage models. If these embedded layers aren’t useful in predicting second stage outputs, then gradient descent optimization will drop them. Because new data can be added with reduced concern of data quality, many different endpoints can be integrated. The advantage of the ability to add many different endpoints to the model is the ability to train models far more quickly for both higher accuracy and with lower requirements on the amount of training data. The Stage (2) models have balanced accuracies of 75-90% across the nine endpoints which is an improvement of 5-10% relative to the simpler Stage (1) Qsar models.

1778 Identifying Contributors to the Response to Endocrine Drug Therapy in Breast Cancer: An In Silico Study
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A number of reports indicate differences in breast cancer presentations between patients of European and those of African ancestry. These include differences in average tumor size, age of disease onset, and degree of aggressiveness. Further, the development of resistance to breast cancer endocrine therapies remain intractable. In this study, gene expression (RNASeq version 2), mutation, and clinical data collected from patients as part of the Cancer Genome Atlas were examined for insights into disparities in responses to endocrine therapy in breast cancer. Notably, expression of the aryl hydrocarbon receptor (Ahr) is suppressed in Black or African-American (BAA). Using Ahr-associated genes sets, gene set enrichment analyses results indicate that two gene sets consisting of genes elevated in endocrine therapy-resistant breast cancer in a mouse xenograft model (Creighton et al., 2008) are enriched in samples from White patients relative to those from BAA patients (p value =0.02, FDR=0.11; p value = 0.04, FDR=0.20). A third gene set of suppressed estrogen receptor (ESR1) targets, i.e. genes down-regulated in an ESR1-negative human breast cancer cell line (Gozgit et al., 2007), was similarly enriched in samples from White patients (p value =0.03, FDR=0.187), Furthermore, using a machine learning approach, a combination of leading edge genes for these three gene sets, along with attributes such as the age at initial diagnosis, menopausal status, race, and hormone and growth factor receptor expression status, were modest predictors of the response to endocrine drug therapy. Pooled gene expression profiles of responders and non-responders to therapy with endocrine drugs (selective estrogen receptor modulators such as Tamoxifen, selective estrogen receptor down-regulators, gonadotropin releasing hormone agonist, and aromatase inhibitors) were subsequently ex-
Evaluating Potential Refinements to Existing Computational Studies of a Select Group of 10 disease categories (including Cardiovascular, Anti-Infective, Central Nervous System, Anesthetic, Gastrointestinal, ACE Inhibitors, and Hypnotics and Sedatives), there were more than 20 new drugs in each in DILIst. DILIst contained a total of 1000 compounds of which 100 were newly added when compared with DILIRank. DILIst also provided the largest number of drugs classified by human hepatotoxicity and covered the broadest range of drug classes to date. It is a great resource for the community to improve DILI research in the areas of elucidation of mechanisms, predictive model development, biomarker identification, and provides additional opportunities to exploit the potential of emerging technologies.

**1781** Computational Studies of a Select Group of Amino Alkylindoles

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Sponsor: R. Bright

Synthetic cannabinoids are man-made chemical toxins used in plant materials and believed to give similar psychoactive effects as natural cannabis. Although early synthetic cannabinoids were used to understand the human endocannabinoid system, they are now drugs of abuse that lead to adverse physical effects when used. The present study employed computational chemistry methods to investigate the structure-activity relationships of five aminoalkylindoles to gain a deeper understanding of the effects synthetic cannabinoids have on the human body. The Australian 09 package was used to model the electronic structures of the compounds. The structures underwent molecular geometry optimization and frequency calculation, and from the output geometries, molecular descriptors were calculated that are related to the electronic distribution, which can indicate the individual compound's ability to interact with biostructures. Molecular descriptors calculated include electrophilicity, chemical hardness, $Q_{el}$, and $E_{HOMO}$. Results indicated that $Q_{el}$, as well as chemical hardness were closely related to the biological activity with R2 values of 0.88 and 0.65, respectively. From these results, it was concluded that the activity could possibly be closely related to the aminoalkylindoles' behavior as hydrogen bond acceptors.

**1782** Evaluating Potential Refinements to Existing Thresholds of Toxicological Concern Values for Environmentally Relevant Compounds

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The Toxic Substances Control Act mandates the US EPA to perform risk-based prioritization of chemicals in commerce and then, for high-priority substances, develop risk evaluations that integrate toxicity data with exposure information. For chemicals where neither in vivo and in vitro studies are available, one approach being considered is a Threshold of Toxicological Concern (TTC)-to-Exposure ratio. The TTC approach has established different levels of human exposure below which there is expected to be a low probability of risk to human health. Within the TTC paradigm, a chemical is assigned to a specific class based upon an evaluation of the chemical structure. In this study, we derived TTC values based on oral (sub)chronic No Observable (Adverse) Effect Level (NO(A)EL) data from the US EPA’s Toxicity Values (ToxVal) database and compared them with published TTC values. We profiled 12,116 unique discrete chemicals present in the ToxVal database into their respective TTC categories using the Kros et al (2004) TTC module within the Toxtree tool. Of these chemicals, 1800 (14.9%) were not considered appropriate for TTC based on exclusion criteria and were removed. The remaining chemicals were assigned to one of the five TTC classes (Cramer structural class I, II, III, containing alerts for genotoxicity and acetylcholinesterase inhibitors [AChEI]). We calculated TTC values for chemicals in Cramer I and III categories as 0.0134 mg/kg bw/day and 0.00199 mg/kg bw/day, respectively. Due to a lack of data we were unable to calculate a TTC value for Cramer Class II chemicals. The TTC value for the Class I chemicals calculated in this study is more conservative than that of Munro et al (1996) (0.07 mg/kg bw/day) and Munro et al (1996) (0.19 mg/kg bw/day). The difference in TTC values from those calculated by Munro et al (1996) could be attributed to a difference in dataset composition as Munro et al (1996) contained a number of food additives in comparison to the ToxVal dataset, which typically comprised food-use pesticide active ingredients. This abstract does not necessarily reflect US EPA policy.
Predicting Neurological Targets of Toxicity Employing the Read-Across Approach of ToxCast In Vitro Data
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Interaction of chemicals with multiple protein targets is the primary reason for triggering an adverse drug reaction. Significant amounts of information about the biological activities of chemicals have already been collected using experimental methods and stored in a variety of public databases. Under the US Environmental Protection Agency’s ToxCast program, the biological activity for thousands of chemicals was screened in hundreds of biochemical and cell-based assays in a high-throughput manner. Using the Gene Ontology database, we identified 123 proteins screened in the ToxCast assays that have neurological functions. Data from these assays were imported into the Organization for Economic Co-operation and Development QSAR Toolbox and used to identify neurological targets for chemicals of interest. Two sets of data were generated: (1) one set coded with a “1” for active compounds and a “0” for inactive compounds was used to classify compounds as active or inactive, and (2) another set composed of the AC15 for only active compounds was used to predict the AC50 values for unknown chemicals. Chemical grouping and read-across within the QSAR Toolbox were used to identify neurologic targets and predict interactions for two psychotic drugs: para-methoxyamphetamine and 4-methylaminorex. Our results demonstrate that ToxCast in vitro screening data and the QSAR Toolbox can be used to identify neurological targets for chemicals that have similar chemicals in the ToxCast database. Cleared, BPFA, Case Number 2018-2711, 25 May 2018.

Looking Under the Hood—Expert Review of In Silico Carcinogenicity Predictions
B. Hansen, and J. Cohen, Gradient, Cambridge, MA.

Alternative testing strategies are seeing increased application in chemical safety assessments across a variety of contexts. However, predictive toxicology tools should not be used as a “black box,” and limitations including “out of domain” results (i.e. no valid prediction can be made) require expert review to make informed hazard and risk conclusions. We evaluated the in silico carcinogenicity predictions for three perfluorinated compounds [Tetradecafluorohexane; CAS 355-42-0; [Hexacosafluorodecane; CAS 307-59-5], and [Perfluoro(1,3-dimethylcyclohexane); CAS 335-27-3] using the freely available Toxtree program as well as the expert-rule based program Derek Nexus. We then applied expert review to the Toxtree program’s assessment of chemical structure and carcinogenic potential. The two perfluorinated alkane chemicals triggered an alert for perfluorooctanoic acid (PFOA), in spite of evidence indicating these compounds are relatively unreactive, and the fact these compounds lack any oxygen or hydroxy functional groups typical of PFOA compounds. Furthermore, this alert was triggered for a series of six additional perfluorinated alkanes (C6-C12, C16, and C24) suggesting that these results are attributable to their common fluorinated cycloalkane substructure. The perfluorinated aryi compound triggered an alert as a polyhalogenated cycloalkane. This alert was triggered for any halogenated aryi with three or more halogens bonded directly to the ring. Yet, neither the supporting mechanistic justification for this specific alert nor the available training data set considered perfluorinated cycloalkanes. Evaluation of these same compounds with the expert-rule program Derek Nexus did not trigger any alerts for carcinogenicity. These findings support our conclusions that the Toxtree carcinogenicity predictions for these three perfluorinated compounds are inaccurate, and highlight the importance of expert review in the application of in silico toxicology tools. The structural alerts we considered for the in silico assessment of these three chemicals have the potential to overgeneralize perfluorinated compounds and propose unlikely toxicological mechanisms.

Application of the SOHN Methodology to Build Accurate hERG Models Using a Combination of Public and Proprietary Data
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The Self Organising Hypothesis Network (SOHN) methodology is a recent modelling approach[1] that has been successfully applied to develop predictive models for the mutagenicity endpoint, which is largely driven by chemical reactivity[2]. Here we show that the SOHN methodology can also be applied, with good performance, to receptor based endpoints like hERG. The predictivity of the resulting hERG models is owed to the SOHN algorithm, the choice of a well-designed pharmacophoric descriptor and the combination of public and proprietary data. We use inhibition of the hERG ion channel to explore such combinations with the aim to develop an accurate model, as this endpoint is a key safety concern in drug development as hERG inhibition has resulted in multiple drugs being withdrawn from the market. We validate the performance of the SOHN methodology and show that combining public and proprietary data sources improves performance due to a better coverage of the target chemical space. The best combination allows for very good hERG predictivity. This demonstrates the suitability of the SOHN methodology for building models for receptor based endpoints. Finally, we show that combining the SOHN models with other statistical models and expert systems, we can leverage additional synergistic effects by taking into account the confidence in the predictions when merging systems. [1] Hanser T, Barber C, Rossler E, Vessey JD, Webb SJ, Werner S: Self-organising hypothesis networks: a new approach for representing and structuring SAR knowledge. J Cheminform. 2014, 6:21.10.1186/1758-2946-6-21.[2] https://www.lhasalimited.org/products/sarah-nexus-model-building.htm.

Computational Analysis of Pre-Clinically Efficacious Drug Molecules without Defined Target Pharmacology

Small molecule drug discovery usually starts with a random screening of large numbers of compounds against either with a validated drug target (target-based drug discovery) or in a physiologically relevant biological system (phenotypic drug discovery). Pharma and biotech industries have, during the past decades, successfully utilized the former approach to discover new drug molecules for a given drug target. However, there are many pharmacology tools working on multiple phenotypic drug discovery programs. The phenotypic screening derived leads have been playing a major role in the discovery of new therapeutics. However, identification of the physiologically relevant targets for these phenotypic drug discovery remain a major challenge. These drug molecules often bind to one or more targets with varied binding strengths. It is difficult to characterize these binding events using conventional experimental methods such as affinity chromatography. Recently, however, this discovery approach is getting significant attention because of the availability of novel advanced in vitro and in silico technologies that can identify novel pharmacological descriptors and understand the physiology of the phenotype. In this work, we have built an efficacious bioactive molecule database (BioactDB) by compiling 3078 pre-clinical and clinical stage drug molecules with no defined pharmacological targets (phenotypic derived drugs). These drug molecules belong to a diverse range of therapeutic classes. Here we are focusing on two classes, namely, 1) oncology (418 drugs) and 2) CNS (511 drugs) for further computational analyses. These drugs, without biological targets, were searched for structural similarity using cheminformatics and machine learning (SVM, RF and NN) based target prediction approaches. This computational approach suggested a testable target hypothesis for some of these clinically efficacious drug molecules that are not known targets. A detailed target analysis on selected drug molecules from BioactDB along with available in vivo data will be presented.

Quantitative Hazard Assessment and Product Scoring Methods for High-Throughput Chemical Alternative Assessments
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Leading consumer product brands are increasingly requiring more quantitative data from suppliers to select safer product and process chemicals. These brands go beyond traditional reactive approaches to meet chemical regulatory compliance by using underlying human and environmental health information in chemical selection. Such proactive approaches enable brands and suppliers to identify controversial, acceptable, and preferred chemicals during product development. Unlike the inherent criteria in most assessment frameworks that rely on qualitative data and assumptions, quantitative methods first score individual SOHNs and products enable more refined comparison and selection of preferred, safer, alternative chemicals and formulations, and hence allow for straightforward defensible decision-making. To this end, the authors developed a quantitative hazard assessment scoring method to complement qualitative assessment frameworks. The method facilitates more efficient and effective alternatives selection, especially at scale using transparent criteria. The method is based on 22 human and environmental health endpoints and physical characteristics. Each endpoint is factored into the method based on hazard rating, type of endpoint (i.e., core endpoints such as
1790 Toxicogenomic-Based Gene Co-regulated Network Analysis for Quantitative Mode-of-Action Assessment of Chemical-Induced Organ Toxicity

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Toxicogenomic data in safety testing represent a critical source to uncover underlying mechanisms of drug-induced toxicities. Co-regulated gene network approaches can organize high dimensional toxicogenomic data, while not being biased by prior gene annotations, and help in identifying novel mechanisms and regulators of toxicity. We applied weighted gene co-expression network analysis (WGCNA) on the publicly available datasets in TG-GATEs on different toxicogenomic model systems, including primary human hepatocytes (PHH), in vivo rat liver and rat kidney. The 3 datasets were independently processed with an unsigned WGCNA analysis that clustered the gene sets into functional modules, representing the bridge between individual gene variations and emergent global properties. The modules serve as dynamic visualization of the transcriptome under experimental conditions (compounds, concentrations and time points). We developed a user friendly tool using the R shiny package for visualization of the toxicogenomic network and analyzing the mechanism of toxicities, called the TXG-MAPr. A dedicated upload function allows to overlay a new set of gene expression data onto the built WGCNA network for the 3 model systems. New Eigengene Scores (EGs) for all modules in the TXG-MAPr are calculated considering the newly uploaded data. Users can analyze dose- and time-response curves, compound correlation plots and functional annotation of the modules to derive mechanistic information of the toxicity. In addition, we included the prediction of transcription factor activities, as well as physical interactions of downstream proteins encoded by the transcriptome, which may be useful in analyzing perturbations triggered by exposure with toxic compounds. In conclusion, with the TXG-MAPr we present an innovative tool that helps in the mechanistic understanding of potential adverse drug reactions that can be used during early drug development. The work received funding from the Innovative Medicines Initiative 2 Joint Undertaking for the TransQST (grant agreement 116030) and eTRANSAFE (grant agreement 777365) projects, which is supported by the EU Horizon 2020 program and EFPIA.

1791 Development of a Generalized Inhalation Model for Use with the High-Throughput Toxicokinetics (httk) Package in R


Currently it is difficult to prospectively estimate human toxicokinetics (particularly for novel chemicals) in a high-throughput manner. The R software package htkk has been developed, in part, to address this deficiency; however, it is limited to oral and intramuscular exposure routes. The purpose of this investigation was to develop a generalized inhalation model for htkk. The structure of the inhalation model was based on a published physiologically based model. The model was evaluated with literature. In total, 42 volatile organic chemicals (VOCs) were identified with sufficient information available in literature (metabolism parameters and concentration vs. time data). Physicochemical data were obtained using the Environmental Protection Agency’s CompTox.
Chemistry Dashboard, while fraction unbound in plasma was calculated with ADMET Predictor™, 9.0. In total, 144 dosing situations were tested (77 in humans, 67 in rats) in plasma, blood, or exhaled breath. Mean (range) molecular weight was 114.99 g/mol (32.04-252.32 g/mol) and mean log P was 2.1 (-0.6-6.1). The slope of the regression line of best fit between log-transformed predicted and measured plasma, blood, and exhaled breath concentrations for combined human and rat data was 0.56 with an R² = 0.50 and a root mean square error (RMSE) = 0.72. Individual species fits were similar. The RMSE of the same data against the identity line was 0.82. The VOCs examined in this investigation represent small, generally more lipophilic molecules. About 3.6% of the data points were censored due to concentrations that were below reasonably measurable limits. Goodness-of-fit values currently indicate moderate model fit to literature data. Future efforts will be focused on identifying trends in model fit relative to chemical properties. This abstract does not necessarily reflect US EPA policy.

1792 Delivering Rapid Computational Profiling of Chemicals by High-Throughput Toxicokinetics

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Department of Defense (DoD) weapon systems require tremendous cradle-to-grave support, including a heavy reliance on hazardous materials that are often unique contaminants with unspecified toxicological categorization. With over one million chemicals in existence and only about 1,000 with occupational exposure limits from well-regarded sources, an automated, rapid assessment of occupational health is necessary for screening chemicals for occupational health purposes. A statistical analysis program in R, htk, was created by the US Environmental Protection Agency (US EPA) to efficiently rank characterized chemicals in order of priority, instead of testing chemicals in a random order. Inputting just a few chemical (CAS number, molecular weight, Log P) and toxicokinetic (intrinsic clearance) parameters allows any chemical to be modeled and compared to other chemicals, establishing a toxicological profile. Parameters for the uncharacterized chemicals were obtained from a toxicological database known as the OECD Quantitative Structure Activity Relationship (QSAR) Toolbox. This toolbox enables predictions about the physicochemical and biological properties of a chemical based upon well-characterized chemicals with known activity and similar molecular structures. The chemical and toxicokinetic parameters plus compositional variables (Number of rings, bonds, heavy atoms, etc), plus estimated parameters from the OECD QSAR Toolbox (Km in 10g fish, Biotransformation, Half-Life, and biodegradability) were then introduced into a random forest model to predict clearance of the uncharacterized chemicals. The overall random forest results had a Root Mean Square Error (RMSE) of 0.5784, Mean Absolute Error (MAE) of 0.4546, and an R² of 0.7091. The plasma and blood concentrations (Cₜₜ) were calculated for all the chemicals, and this parameter was used to predict concentrations in vivo that cause biological activity (ACₜₜ). As the list of uncharacterized chemicals is studied further, warfighters will greatly benefit in understanding the effects of specific chemical exposures with the aim to derive mitigation strategies.

1793 An Integrated Testing Strategy Assessment Incorporating the Genomic Assay SENS-IS for Skin Sensitization

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The move towards animal-free testing methods for assessing the toxicity potential of chemicals has led to the development of frameworks such as the adverse outcome pathway (AOP) that can characterize the key events that lead to an adverse outcome. Skin sensitisation is one such endpoint that has been prioritized by the Organisation for Economic Co-operation and Development (OECD) and other public agencies. It is also one of the few adverse outcomes for which an OECD-approved AOP exists and assays measuring its key events (KEs) have been developed. Based on work done by Joanna Jaworska et al. (doi: 10.1007/s00204-015-1634-2), we implemented an Integrated Testing Strategy (ITS) for skin sensitisation. It replaces the in vivo Local Lymph Node Assay (LLNA) assay, which has been used as a proxy for skin sensitisation in humans. The defined approach uses a Bayesian network which predicts the skin sensitisation potential of a substance by combining experimental results from the 3 OECD-approved skin sensitisation assays (direct peptide reactive...
multivariate Cox regression model further confirmed standard regimens as a good predictor of ATDH. The nomogram illustrated that standard regimens was the largest contributor to ATDH, followed by age, weight, gender, past medical history of liver diseases, medical history of diabetes mellitus, drink and smoke. The performance of each classification model was evaluated by the area under the Receiver Operating Characteristic (ROC) curve (AUC). The SVM models achieved the best performance with an average AUC of 0.84, followed by NB (0.83), and RF (0.79). The survival analyses and ATDH prediction models established in this study could be applied to help clinicians evaluate the TB patients' conditions to choose suitable treatment regimens.

1796 PharmOmics: A Species- and Tissue-Specific Drug Signature Database for ADR Prediction and Drug Repurposing


Elucidating species- and tissue-specific molecular actions of drugs can provide systems-level insights into drug toxicity and therapeutic activities. However, systematic efforts in cataloging the molecular pathways influenced by individual chemicals in a species- and tissue-specific manner remain limited. Here, we present a comprehensive drug knowledgebase, PharmOmics, comprised of genomic footprints derived from meta-analysis of microarray and sequencing data relevant to drugs/substances from tissues and cell cultures derived from human, mouse, and rat samples in GEO, ArrayExpress, TG-GATEs and drugMatrix data repositories. Using multi-tissue 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 demonstrate the potential of PharmOmics to facilitate drug repurposing using a network-based disease-drug matching algorithm and to predict adverse drug reaction (ADR). Using the uric acid increment ADR as an example, our experimental validation supports the accuracy of PharmOmics in making novel predictions. By leveraging tissue- and species-specific analysis, PharmOmics has advantages over the commonly used cell line-based drug signatures to facilitate drug development.

1797 XomeTox—Evaluating Multi-omics Integration for Assessing Rodent Thyroid Toxicity

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Exposure to chemicals triggers a series of effects at the molecular level. Regulatory pathways involved in such responses exhibit changes of levels, interactions, and feedback loops of biomolecules of different types that are active in complex networks. Different ‘omics techniques are essential for measuring responses in an untargeted manner on the molecular level. Importantly, a single ‘omics technique will detect biomolecules of one type and thus captures changes only for a small subset of the components of a particular pathway. Therefore, applying single ‘omics analyses in response to a toxicant in a noncontinuous design led to the identification of biomarkers for certain exposures but not to a systemic understanding of toxicity pathways or adverse outcome pathways (AOPs) in the past. Also, this incomplete representation of pathways in single ‘omics data limits the ability to discriminate adaptive from adverse molecular responses. We propose that a substantial improvement in detecting the pathway response to a toxicant can be achieved by using multi-omics data in a time- and concentration-resolved design. We will initially compile a collection of existing multi-omics data sets, including the integration of BASF generated metabolomics data, to evaluate integrative multi-omics analysis approaches and to identify criteria for an optimal design of a multi-omics toxicity study. Based thereon, we will perform a rat study focusing on direct and indirect thyroid toxicity to generate transcriptionomics, proteomics, and metabolomics data sets. We aim to (a) detect toxicant-triggered re-wiring of regulatory networks and changes of master-regulators, (b) evaluate whether time- and dose-resolved multi-omics data enable the prediction of adversity, (c) evaluate whether deep learning may assist in discriminating adaptive vs adverse perturbations of regulatory networks, and (d) determine to what extent each ‘omics experiment contributes to the predictive power of such an approach. XomeTox is funded by CEFIC Long Range Research Initiative.

1798 Comparison of Impacts of Cigarette Smoke and High-Fat Diet on Transcriptomes of Principal Organs in ApoE Knockout Mice


High-fat diet (HFD) and cigarette smoking are risk factors for atherosclerosis progression. To understand the impacts of HFD intake and mainstream cigarette smoke (MCS) inhalation on the progression of atherosclerosis, we conducted a subchronic MCS inhalation study with HFD feeding for 13 weeks using female apolipoprotein-E knockout (ApoE) KO mice. Pathologically, HFD feeding greatly accelerated the progression of atherosclerosis compared with MCS inhalation, suggesting differences in the contributions of diet and cigarette smoke to atherosclerosis progression in ApoE KO mice. To further investigate these differences, we analyzed the effects of 13-week HFD feeding and MCS inhalation on transcriptomic changes in the lungs and livers of the mice. Differentially expressed genes were analyzed by Ingenuity Pathway Analysis software to elucidate the biologically relevant canonical pathways. In the lung, HFD feeding did not perturb any canonical pathways, while MCS inhalation significantly perturbed NRF2-mediated oxidative stress response and inflammatory responses. In the liver, HFD feeding and MCS inhalation significantly perturbed different pathway-related gene expressions: HFD feeding significantly perturbed inflammatory responses and cholesterol biosynthesis, while MCS inhalation perturbed not only xenobiotic-activated pathways, but also lipid metabolism-related pathways, such as LXR/RXR pathway. Overall, HFD feeding and MCS inhalation caused differential perturbation of canonical pathways in the lung and liver. These data indicate that diet and cigarette smoke contribute to atherosclerosis progression through differential effects on organ transcriptomes in ApoE KO mice.

1799 Toxicogenomics: From Snapshot Observations towards Dynamic Response Modelling


In chemical risk assessment the integration of knowledge about a chemical's mode of action (MoA) is under way, meaning that we move from an observational perspective, considering only concentrations leading to death after pre-defined exposure durations, towards a mechanism-based perspective, where knowledge about time courses of molecular reactions and respective consequences for an organism's health will become decisive. Toxicogenomic methods are enabling us to generate such information on molecular responses upon chemical treatment. However, aggregated effect measures, such as EC50 values, are often not retrievable from those data due to limitations in experimental designs not capturing dependencies of molecular responses on factors, such as exposure concentrations or durations. A comparison of molecular response fingerprints between compounds as well as an extrapolation or general assessment is therefore difficult (Schüttler et al. 2017, https://doi.org/10.1093/toxsci/kfx045). Based on previous achievements on modeling of dose response relationships for different omics data sets (Semtanová et al. 2015, https://doi.org/10.1002/etc.3025) and experiences with zebrafish embryo toxicogenomics, we developed an experimental design for studying the concentration and time dependent changes in the zebrafish embryo transcriptome after chemical treatment. The investigated concentration ranges were thereby anchored to phenotypic lethal and sublethal observations. In order to handle and analyse such data, we developed an analysis pipeline and regression models for description and visualization of the expression behaviour of each gene over time and dose which enables extrapolation and the retrieval of model parameters for comparison of genes, pathways, and compounds. The approach was applied in a case study on the exposure of zebrafish embryos to three selected model compounds, two with similar and one with distinct MoA. Our results demonstrate that every compound causes its own dynamic fingerprint, which strongly depends on the compound's toxicokinetics. Nevertheless, similarities, MoA-specific responses, as well as potential key events indicating a general response to stress could be identified and quantitatively described with the developed regression models. Having these models for every single response on the transcriptome, now allows predictions on mixture effects on the single gene level, the pathway level, as well as on the whole transcriptome. The reliability and robustness of such predictions has to be investigated and is currently subject of our research.
High-throughput in vitro methods are being increasingly used for assessing the safety of chemicals. Two aspects of hazard assessment are critical: identification (i.e., what targets, pathways or processes does a chemical perturb) and potency estimation (i.e., at what concentration or dose does the chemical perturb these biological processes?). New experimental methods for running whole genome high-throughput transcriptomics (HTT) experiments are making it possible to simultaneously assess many chemicals in dose response to aid both hazard identification and potency estimation. Here we describe results of a pathway modeling effort in which over 2000 chemicals were run in an HTT screen in MCF-7 cells using the BioSpyder TempO-Seq platform (8 test concentrations / chemical). The raw count data was processed using the DESeq2 R-package to generate log2 fold change data for each chemical-concentration sample, for each of 3 biological replicates. Multiple pathway-level concentration response modeling methods were compared. These include variants of GSEA (Gene Set Enrichment Analysis), and a simple comparison of the average fold changes of genes in and out of a pathway (In-Out). Different filters for significance were used (filtering at the gene vs. pathway level). Concentration modeling was performed using the ToolCast Pipeline (tcpil) software. Method performance was evaluated using reference chemicals for multiple molecular targets, curated from the literature into a database called RefChemDB. Three main results are (1) The simple In-Out and the GSA variant (Gene Set Variation Analysis) of GSEA were able to detect appropriate reference chemical / pathway interactions with potencies similar to what is seen for in vitro target-based assays; (2) For many chemicals, many pathways are activated in a non-specific way, indicating overall cell stress; and (3) Further research will be required to select an optimal method for assessing pathway level potencies from HTT data. This abstract does not necessarily represent US EPA policy.
Breast cancer is a leading cancer type and one of the major health issues faced by women around the world. Some of its major risk factors include body mass index, hormone replacement therapy, family history and germline mutations. Of these risk factors, estrogen levels play a crucial role. Among the estrogen receptor (ER) family of estrogen receptors, the ERα is known to interact with tumor suppressor protein, p53 directly thereby repressing its function. Previously, we have studied the impact of deleterious breast cancer-associated non-synonymous single nucleotide polymorphisms (nsSNPs) rs11540654 (R110P), rs17849781 (P278A) and rs28934874 (P151T) in p53 gene on the p53 DNA-binding core domain. In the present study, we aim to analyse the impact of these mutations on p53-ERα interaction. To this end, we have modelled the full-length structure of human p53 and validated its quality using PROCHECK and subjected it to energy minimization using NOMAD-Ref web server. Three-dimensional structure of ERα activation function-2 (AF-2) domain was downloaded from protein data bank. Interactions between the modelled native and mutant (R110P, P278A, P151T) p53 with ERα was studied using ZDOCK. Machine learning prediction on the interactions was performed using Weka software. Results from the protein-protein docking showed that the atoms, residues and solvent accessibility surface area (SASA) at the interface was increased in both p53 and ERα for R110P mutation compared to the native complexes indicating that the mutation R110P has more impact on the p53-ERα interaction compared to the other two mutants. Mutations P151T and P278A, on the other hand, showed a large deviation from the native p53-ERα complex in atoms and residues at the surface. Further, results from artificial neural network analysis showed that these structural features are important for predicting the impact of these three mutations on p53-ERα interaction. Overall, these three mutations showed a large deviation in total SASA in both p53 and ERα. In conclusion, results from our study will be crucial in making the decisions for hormone-based therapies against breast cancer. We have developed the US FDALabel database as a web-based application, containing over 100,000 drug labeling documents from US FDA's SPL archive. US FDALabel allows the public to perform customizable (any combination of sections, document types, market categories, and other information), full-text searches of product labeling on a relational oracle database. A new version of FDALabel (v 2.3), available at Amazon Cloud: https://ncr-crs.fda.gov/fdalabel, was developed to search human prescription drug and biological product labeling and human over-the-counter (OTC) drug labeling. To demonstrate the FDALabel database's utility, we have selected study cases including a pharmacogenomics study for Precision Medicine and an ADR (Adverse Drug Reaction) study that applied Medical Dictionary for Regulatory Activities (MedDRA) standard terminologies. We identified 224 drugs with 289 drug-biomarker pairs across different therapeutic areas such as oncology (103), psychiatry (33), and infectious diseases (32). We also found severe ADRs were prevalent in MedDRA System Organ Classes such as Nervous system disorders, Psychiatric disorders, and Cardiac disorders. The FDALabel database search offers the public a useful and regulatory reviewers an efficient and user-friendly means of accessing and searching the large amount of information contained in drug labeling. An Amazon Cloud version of FDALabel (v 2.3) supports and promote translational medicine and regulatory science by employing advanced computer technologies to deliver end-users a reliable, effective, and efficient search tool.

A narrow range of endogenous retinoic acid (RA) levels is required for proper anteroposterior patterning of the early embryo. Insufficient or overabundant RA levels disrupt the expression of HOX and other genes important for axis patterning and can lead to birth defects such as cleft palate. We hypothesized that the expression of development marker genes is measurably altered by exogenous concentrations of all-trans retinoic acid (ATRA) that affect endogenous RA signaling. To confirm molecular markers of altered embryogenesis, a pilot study was conducted in differentiating endoderm exposed to ATRA. Induced pluripotent stem cells derived from adult female fibroblasts were directed to a differentiating endodermal trajectory using low serum and activin. Differentiating cells were then exposed daily to five concentrations of ATRA (0.001 to 10 µM) or 0.1% DMSO control and collected at three timepoints (6h, 96h, and 192h) for high content imaging (HCI) and RNA-sequencing analysis. HCI analysis quantitated the cell counts and percentage of cells that were dead, metabolically active, or expressing FOXA2 (a definitive endoderm marker). These results were compared to the differentially expressed genes (DEGs; padj < 0.05 and abs(log fold change) > 1) at each time and concentration. Using system trajectory and dynamics analysis of the DEGs, a tipping point defined by the persistence of effects at both 96h and 192h timepoints was identified at 0.01 µM. Global gene expression at concentrations above and below the tipping point followed two diverging trajectories reflected in a principal components analysis. The tipping point was identified at concentrations above 0.01 µM resulting in an increase in cell counts and reduced FOXA2 expression compared to time-matched DMSO controls. Approximately 100 genes were selected as developmental markers to include the primitive streak, definitive endoderm, yolk sac endoderm, foregut, midgut, and hindgut. The expression profile of these marker genes suggested that cells exposed to concentrations above 0.01 µM shifted from an anterior to posterior molecular phenotype compared to cells exposed to concentrations below the tipping point. The molecular markers of this phenotypic shift will be used to establish a testing platform to screen chemicals for potential disruption to early embryonic development through altered RA signaling. This work does not represent US EPA policy.

There is a need for approaches to understand the biological mechanism of adverse outcomes and human variability in response to environmental chemical exposure. The US EPA Adverse Outcome Pathway Database (AOP-DB) is a database resource that combines different data types (AOP, gene, chemical, disease, pathway, orthology, and ontology) to characterize the impacts of chemicals to human health and the environment, and serves as a decision support tool for case study development. Here we present an updated version of the database, which includes an increased number of adverse outcomes and corresponding key events derived from updated feeds from the AOP-Wiki (https://aopwiki.org/), updated disease, phenotype, and ontology information, as well as improved integration with the AOP-DB web user interface. We illustrate these updates and the utility of the AOP-DB through three guided tutorials which provide the user with opportunities to explore computationally how chemical-gene molecular key events in adverse outcome pathways link to adverse disease, species-specific factors and population-level health outcomes. This abstract does not reflect US EPA Policy.

The Aryl Hydrocarbon Receptor (AhR) is a ligand-inducible transcription factor that regulates genes involved in a variety of physiological functions in response to the potent and persistent environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Assembly of AhR regulatory networks is critical for understanding the intracellular events that lead to tissue-specific adverse health effects upon chemical exposure. However, the precise sequence of DNA binding sites of the AhR, as well as its extended AhR regulon, has yet to be elucidated. In this study, we applied...
a supervised machine learning model to predict direct AhR binding sites. We predicted functional AhR binding sites in the accessible DNA regions in the HepG2 cell line from a model trained on AhR ChIP-seq data in MCF-7 cells. Moreover, we predicted the AhR ChIP-seq peaks across cells from a model trained on an integrated dataset including DNA sequence, open chromatin and histone methylation profiles. By combining our predictive model with RNA-Seq analysis of TCD-affected HepG2 cells, we mapped the AhR binding sites to TCDD-responsive genes, and reconstructed the AhR regulatory network in the HepG2 cell line. Our work provides an approach for reconstruction as well as analysis of AhR regulatory cascades in multiple tissues by combining accurate prediction of AhR binding sites and mapping AhR binding sites to proximal and distal target genes

1809 Functional Consequences of miRNA Regulation by Polycyclic Aromatic Hydrocarbons in 3D Human Lung Epithelial Cells

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Polycyclic aromatic hydrocarbons are a class of contaminants ubiquitous in the environment from the incomplete combustion of fossil fuels. Many PAHs have been identified as procarcinogenic, which are metabolized to form DNA adducts; however, other mechanisms also may contribute to toxicity and help explain differences in toxicity across the wide class of compounds. This study focused on the role of miRNA in regulating toxicity by benz(a)pyrene (BAP) and dibenzofuranylene (DBF) on the 3D human bronchial epithelial cells (HBE). miRNA are short (≤22 nt), non-coding RNA molecules that post-transcriptionally regulate gene expression. Cells were exposed to 500 µg/ml BAP and 10 µg/ml DBF for 48 hrs and samples were collected for RNA isolation and parallel analysis of miRNA and mRNA by RNA sequencing using Illumina HiSeq3000. Significant (q<0.05) differentially expressed miRNA and mRNA were analyzed in an anti-correlated fashion in Bioinformatics Resource Manager to identify miRNA-mRNA interactions and visualized as networks in Cytoscape to identify patterns of regulation reflecting a response to PAHs. DBC treatment showed more regulation of unique miRNA with 53 significantly down- and 46 up-regulated miRNAs, compared to BAP’s 14 down- and 35 up-regulated miRNAs. These miRNA targeted 546 up- and 654 down-regulated genes significant in the DBC dataset, and 176 up- and 750 down-regulated in the BAP. miRNA uniquely up-regulated in BAP were linked with a more significant response in cell adhesion and developmental processes. In DBC, up-regulated miRNA showed more significant response overall in regulation of cell cycle, translation, and apoptosis. Processes perturbed by down-regulated miRNA were less consistent. These data are the first to describe the role of miRNAs as regulators of PAH toxicity in primary human 3D HBEC and could represent important mechanisms associated with PAH-mediated lung disease and cancer in humans.

1810 Dysregulation of ID3-Regulated Transcriptomic Signatures by Polychlorinated Biphenyls

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Environmental factors, including polychlorinated biphenyls (PCBs), are considered to be involved in hyper-proliferative vascular remodeling because genetic makeup can only explain about 10% of severe PAH cases. The molecular mechanism associated with PCBs role in lung toxicity is unclear. Our previous studies demonstrated PCB153 mediated vascular endothelial dysfunction and activated the inhibitor of differentiation protein 3 (Id3). To determine the molecular mechanism by which Id3 contributes to hyper-proliferative endothelial cells, we investigated Id3 transcriptional reprogramming using ChIP-Seq and RNA-Seq technology. Stable ectopic expression of Id3 in lung endothelial cells contributed to endothelial-mesenchymal transition (EndMT), cell proliferation, and cell migration. Id3 overexpressing cells showed the loss of VE-cadherin and gain of MMP9 and vimentin, which are markers of EndMT. PCB153 also increased phosphorylation of Id3 in lung endothelial cells. Our ChIP-Seq data show that Id3 binds to a subset of 2834 target genes. Comprehensive motif analysis of ChIP-Seq data using the MEME Suite software toolkit revealed that Id3 bound to the GAGAGAGA motif sequence on genomic DNA. We also show a significant preference of Id3 binding to motifs associated with transcription factors IRF1, BC11A, IRF4, PRDM1, FOXJ3, SMAD4, 2B1B6, GATA1, and STAT2. Using an integrative approach of ChIP-Seq and RNA-Seq data, we identified 19 genes whose promoter region was bound by Id3 and RNA was differentially expressed in Id3 overexpressing cells. In summary, our data demonstrated that PCB153 and/or Id3 induces proliferation of lung endothelial cells via transcriptional reprogramming. Discoveries from these findings will lay the necessary groundwork for testing the efficacy of Id3 antagonists for the prevention and treatment of pathological vascular remodeling as well as provide a new paradigm by which PCBs may contribute to lung vascular toxicity.

1811 Repurposing Immortalized Cell Line-Based Transcriptomic Profiling Assays for Drug-Induced Liver Injury with a PRank Method

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In vitro Toxicogenomics (TGSTs) as an alternative means to animal studies holds great promise in risk assessment. However, TGx studies often suffer from limited sample size and few available cell types. To investigate the potential of repurposing accumulative transcriptomic profiles data of immortalized cell lines in risk assessment, we carried out a comprehensive assessment of the transferability between transcriptomic profiles from three cancer lines (HL60, MCF7, and PC3). These three lines were compared in Connectivity Map (CMAP) and in toxicogenomic datasets (human primary hepatocytes and rat in vivo repeated dose) from the Open Toxicogenomics Project-Genomics Assisted Toxicity Evaluation System (TG-GATES) database using our developed Pair Ranking (PRank) method. A moderated concordance was observed in HL60 versus human primary hepatocytes (PRank score = 0.70), suggesting the two cellular assays are potentially interchangeable. The suboptimal concordances between rat in vivo repeated dose and cancer cell lines were markedly improved by 10%, when limiting the compounds causing drug-induced liver injury (DILI) endpoints and a gene list of DILI predictive toxicogenomics space (PTGS). Furthermore, some toxicity related pathways including PPAR signaling pathways, and fatty acid-related pathways were consistently perturbed across the assay systems, confirming our previous finding that assay transferability is biological process specific. Also, the extrapolation ability for differentially expressed genes (DEGs) of 304 immune-related states among the assay systems were evaluated, suggesting the preservation of immune-related transcriptional features is assay dependent. In conclusion, these comparisons suggested a great potential of repurposing transcriptomic profiling assays of immortalized cell lines in DILI.

1812 Transcriptomic Approach to Predicting the Impact of Cigarette Smoke on Morphological Changes in Human 3D Bronchial Tissue


The respiratory tract acts as the first protective barrier against inhaled xenobiotics, whereas excessive responses induced by the habitual inhalation of airborne materials can impair barrier function, potentially leading to abnormal morphological changes in respiratory tract tissues. These alterations are found in cigarette smoke (CS)-induced respiratory diseases; however, the precise mode of action (MoA) of these changes remains unknown, possibly because of complexity. Reconstructed three-dimensional (3D) cultures of bronchial epithelial cells are considered useful for understanding the MoA because of their resemblance to human tissues. In addition, comprehensive investigation via transcriptomic analysis could provide insights into the MoA. In this study, we repeatedly exposed 3D human bronchial tissues to EGf, IL-13, and TGF-β for 2 weeks to induce epithelial hyperplasia, goblet cell hyperplasia, and epithelial-mesenchymal transition, respectively, and each morphological change was confirmed by immunostaining. We then performed microarray analysis to investigate the global gene expression profile in the tissues after exposure to each inducer for 4 hours and 2 weeks, followed by the detection of differentially expressed genes (DEGs). The gene expression profile of tissues exposed to CS for 4 hours was also obtained, and then we identified the common DEGs between CS and each inducer, which we named “key genes” (KGs). We found that the majority of KGs were common to both CS and EGf exposure, suggesting that EGFr activation is a dominant effect of CS exposure. Chronic obstructive pulmonary disease (COPD) is a major CS-related respiratory disease exhibiting an increased presence of abnormal cell morphology. Therefore, we further investigated the utility of multi-class classification with KGs for COPD prediction by using publicly available microarray datasets of lungs from healthy non-smokers, healthy smokers, and ex-smokers with COPD. The accuracy of prediction using KGs was higher than that using randomly selected genes. Overall, the transcriptomic analysis suggested that CS-induced respiratory diseases are dominantly coupled to proximal and distal target genes including accurate prediction of AhR binding sites and mapping AhR binding sites to TCDD-responsive genes, and reconstructed the AhR regulatory network in the HepG2 cell line. Our work provides an approach for reconstruction as well as analysis of AhR regulatory cascades in multiple tissues by combining accurate prediction of AhR binding sites and mapping AhR binding sites to proximal and distal target genes.
1813 Profiling of Opioid Information Using FDALabel Database

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The opioid epidemic has drawn national attention and has been officially declared a state of emergency in the US. ~100 million Americans experience chronic pain resulting in an estimated $560-635 billion/year economic burden. As public awareness and education improve, the scope of this epidemic continues to grow indicating that more information and action is needed. US FDA-approved drug labeling contains a complete set of opioid drug products and can be used as a reliable source to aid in understanding the mechanisms and characteristics of opioids. FDALabel Database is a publicly available web application that allows for customized searches against the entire text of drug labeling. To demonstrate FDALabel’s utility, a case-study was designed to collect information from opioid drug products. Queries were performed based on the specific opioid-related pharmacologic classes, chemical structures, DEA schedules, MedDRA terms, labeling sections, and drug interactions. Using this approach, 12 Pharmacologic Drug Classes and 32 active ingredients associated with either opioid antagonists or agonists were identified. The identified drugs are indicated for analgesic, gastrointestinal, addiction, or opioid overdose treatment. Of the 32 drugs, 24 have Boxed Warnings (BW), 22 are controlled substances, 14 are classified as CII (high potential for abuse), and 11 are in the Full Opioid Agonist pharmacologic class. Twenty-seven different instances of drug-drug interactions were also identified. The top 10 occurring MedDRA preferred terms in BW, which correlate to adverse reactions, are Death, Overdose, Dependence, Substance abuse, Depression, Respiratory depression, Nervousness, Coma, Withdrawal syndrome, and Drug interaction. The ever-growing epidemic of opioid abuse is of great interest to the US FDA, which is committed to advancing efforts to address the crisis of misuse and abuse through awareness, education, and research. Here, we provide a comprehensive overview of current opioid information using US FDA-approved drug product labeling to support drug safety research, and for the advancement of opioid abuse and addiction prevention.

1814 The Comparative Toxicogenomics Database (CTD): Mechanism Meets Exposure Science Illustrated through a Bisphenol A-Diabetes Case Study

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The Comparative Toxicogenomics Database (CTD; http://ctdbase.org) is a premier public resource that illuminates the molecular mechanisms by which environmental exposures affect human health. CTD provides manually curated interactions for chemical-gene, chemical-phenotype, chemical-disease, gene-disease, and population-based exposure associations. All data is curated using community-accepted ontologies and controlled vocabularies, providing contextualization and allowing seamless integration across curated content as well as with external data sets to make novel inferences. This information can be explored with user-friendly query and analytical tools to generate testable hypotheses for environmental diseases. CTD includes data from over 130,000 scientific articles, describing more than 2.3 million interactions for 15,600 chemicals, 46,600 genes, 4,300 phenotypes, 7,200 diseases, and 2,100 exposure events for over 580 taxa. We demonstrate how users can access CTD to learn about any chemical, gene, phenotype, disease, or exposure study. Integration of all four modules allows adverse outcome pathways (AOP) for systems toxicology applications to be constructed, from molecular initiating events to population-level health outcomes. As a case study, we demonstrate how to construct mechanistic pathways relaying a putative role for the consumer-exposed product bisphenol A in type 2 diabetes and discover a set of prioritized genes as potential mediators, with pathways correlated at multiple data levels (gene-disease, gene-GO, chemical-phenotype, chemical-anatomy, and population studies). This showcases how users can leverage CTD’s content for any chemical-of-interest to easily generate putative mechanistic AOPs for testing, refinement, and validation in systems toxicology applications.

1815 Pharmacokinetic Models for Quantifying Mother-to-Offspring Transfer of Lipophilic Chemicals


Lipophilic chemicals, which can accumulate in a woman’s body fat due to environmental exposure over the course of many years preceding and during pregnancy, may be transferred to her child during gestation and, after birth, through her breast milk. Because of the bioaccumulation of such compounds in fat and their subsequent partitioning into breast milk lipids, a breastfeeding infant may have an average daily exposure per unit of body mass (mg/ kg/d) that greatly exceeds that of the mother. Thus, nominal maternal exposures occurring in animal studies may not be adequate surrogates for the exposures experienced by developing infants. Furthermore, exposure duration and regimen for developmental animal studies vary tremendously. Maternal laboratory animals are generally exposed to a chemical for a relatively brief time before, during, and/or after pregnancy, whereas human mothers may be exposed for a much longer time encompassing all these periods. In order to quantitatively analyze these issues, we developed a pharmacokinetic (PK) model for lipophilic chemicals in humans, rats, and other animal species that addresses mother-to-offspring transfer of these chemicals during the critical periods of gestation and early post-natal development. This model can be used to estimate instantaneous body burdens (in mg of chemical per kg of body mass) in the mother, fetus(es), and infant(s) based upon gavage or intravenous dosing or dosing through diet. Using our model, we demonstrated that breastfeeding infants tend to have higher average daily exposures to 2, 2′, 4′, 4′, 5′-hexachlorobiphenyl (PCB 153) than their mothers. Also, by considering various dose metrics, we demonstrated a method for computing human equivalent doses for points of departure identified in developmental animal studies of PCB 153. Then, by rank-ordering these human equivalent doses, we showed how one can identify those adverse outcomes most sensitive to chemical exposure.

1816 Integration of Food Animal Residue Avoidance Databank (FARAD) Empirical Methods for Drug Withdrawal Interval Determination with a Mechanistic Population-Based Interactive Physiologically Based Pharmacokinetic (iPBPK) Modeling Platform: Example for Flunixin Meglumine Administration

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Violative chemical residues in animal-derived food products may cause adverse health effects to consumers, affect food safety globally and impact on the trade of international agricultural products. The Food Animal Residue Avoidance Databank (FARAD) has been developing scientific tools to provide appropriate withdrawal interval (WDI) estimations after extralabel drug use in food animals for the past three decades. One of these tools is physiologically based pharmacokinetic (PBPK) modeling, which is a mechanistic-based approach that can be used to predict tissue residues and WDIs. However, PBPK models are complicated and difficult to use by non-modelers. Therefore, a user-friendly PBPK modeling framework is needed to move this field forward. Flunixin was one of the top five violative drug residues identified in the US from 2010 to 2016. The objective of this study was to establish a web-based user-friendly framework for the development of PBPK models for food animals. Specifically, a new PBPK model for both cattle and swine administered flunixin meglumine was created. The determination coefficients of model validation were higher than 0.90 (cattle: 0.92; swine: 0.96), indicating model validation. Approaches that breastfeeding infants tend to have higher average daily exposures to 2, 2′, 4′, 4′, 5′-hexachlorobiphenyl (PCB 153) than their mothers. Also, by considering various dose metrics, we demonstrated a method for computing human equivalent doses for points of departure identified in developmental animal studies of PCB 153. Then, by rank-ordering these human equivalent doses, we showed how one can identify those adverse outcomes most sensitive to chemical exposure.
Physiologically Based Pharmacokinetic (PBPK) Modeling of Mirtazapine in Cats

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Within the veterinary companion animal realm, physiologically-based pharmacokinetic (PBPK) models have been focused toward the canine population, while PBPK models for the feline population have not previously been represented in the literature. Cats possess several unique physiologic differentiations regarding metabolism and transport of certain drugs, including known genetic abnormalities in UGT1A6 and ABCG2 (leading to alterations in glucuronidation and drug transport, respectively), which can often result in severe drug toxicities. Thus, an unmet need exists for feline-specific PBPK models to investigate the dosing and pharmacokinetic profiles of therapeutic drugs used for treating feline disease. Mirtazapine (MTZ), a noradrenergic and specific serotonergic antidepressant, was selected for study as it is commonly used in veterinary medicine as a treatment for nausea and vomiting associated with chemotherapy, pancreatitis, and chronic kidney disease in cats. Based on available data and relevant target tissues, a six-compartment oral dosing PBPK model was developed using the software MCSim. Physicochemical parameters characterizing the ADME properties of mirtazapine (Vmax, Km, tissue partitioning, etc.) were obtained via human- and mice-based studies from the literature and scaled to feline values where appropriate, and anatomic (weight & volume) tissue data were obtained directly from feline necropsies. The model was parameterized using Bayesian inference via a Markov chain Monte Carlo methodology and population pharmacokinetics were realized using Monte Carlo simulations. Results from a typical individual simulation of a 1.88 kg cat, which virtually all available experimental data points, are in agreement with the literature. Results from this study demonstrate a nascent computational framework for investigating drug disposition in cats that may enable a predictive approach for better informing feline drug dosing protocols, especially for cases where drug disposition may be impacted, such as in chronic renal failure or liver disease.

Improved Prediction of Tissue and Urine Concentrations of 2-Phenoxyethanol and Its Metabolite 2-Phenoxyacetic Acid in Rat and Human after Oral and Dermal Exposures via GastroPlus Physiologically Based Pharmacokinetic Modeling

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2-Phenoxyethanol (PHE) is a preservative widely used in various products for personal care and cosmetics. In rats, PHE has been confirmed to be primarily metabolized to 2-phenoxyacetic acid (PhAA). At high dose levels (>400 mg/kg/day) with subchronic or chronic exposures, PHE has been shown to induce hepatotoxicity, renal toxicity, and hemolysis in multiple species (such as rats). Of the numerous metabolic pathways associated with PHE metabolism, the succinyl-CoA:3-hydroxy-3-methylglutaryl-CoA thiolase (HMGCST) pathway results in the formation of PhAA. In the current work, a PHE- and PhAA-based PBPK model can only estimate tissue concentrations of total parent PHE plus its metabolite PhAA individually by tissue and species after oral or dermal exposure. In the current work, a PHE- and PhAA-based PBPK model was initially built in rats via GastroPlusSM software and validated using plasma levels of PHE and PhAA and urinary levels of PhAA obtained from rats intravenously administered 2 mg/kg PHE. This PBPK model was then applied to rat and human exposure scenarios to predict the tissue concentrations of PHE, PhAA, and urinary PhAA. The results showed that predicted tissue concentrations of PHE or PhAA were lower than the experimental tissue concentrations based on total radioactivity (e.g., at 1 hr time point, the experimental plasma concentration of radioactivity (presumed PHE) was more than 10-fold higher than the predicted corresponding concentration of PhE, likely due to PhAA metabolite formation). The predicted urinary concentrations of PhAA were similar to experimental urinary radioactivity concentrations. With this improved model, the tissue concentrations of PHE and PhAA and urinary concentration of PhAA from subchronic and chronic exposures (either oral or dermal) can be predicted and applied for the toxicity risk assessment of PHE.

Carcinogenic Alterations of Mouse Tissue-Derived Organoids by Chemical Treatment

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We previously reported that mouse normal intestine-derived organoids, to which a single exposure to the carcinogenic agent, Apc−/−, p53−/− or Pten−/− knockout mice have been introduced in vitro, showed tumorigenicity after subcutaneous injection to nude mice. In the present study, we examined morphological alterations of mouse organoids treated short-time in vitro with several genotoxic chemicals and injected to nude mouse subcutis thereafter. Treatments with ethyl methanesulfonate (EMS), 10 μM m7G, and 10 μM 200 μM of C78L8/16 mice with KrasG12D mutation and acrylamide (AA) at 1.4 Mm to lung organoids of BALB/c-p53 (+/+ or (+/-) mice for 24 hrs were repeated three times of passages of the organoids, followed by their subcutaneous injection to nude mice. (Results) Treatment with EMS demonstrated subcutaneous nodules over 10 mm in major axis with histological characteristics of carcinomas and AA showed histological characteristics of adenocarcinomas, multilayered and/or invasive futures of epithelia, in nude mice. It was found that macroscopic and/or histological carcinogenic alterations of mouse tissue-derived organoids treated with chemical carcinogens were induced in the nude mouse subcutis. We are now investigating the impacts of other carcinogens and non-carcino-gens, as well as those of host strain differences, on the morphological alterations of the tumors.

Modeling Genetic Diversity of Dioxin Disposition in the Liver with a Genetically Diverse Panel of Mouse Models

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The aryl hydrocarbon receptor (AhR) is a ligand activated transcription factor whose activation results in the induction of multiple downstream genes, including CYP1A1 and 1A2. The prototypical ligand for AhR is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The induction of CYP1A2 is liver-specific and is responsible for additional sequestration of TCDD in the liver. Exposure to TCDD can result in AhR-pathway-mediated hepatotoxicity in animals and humans, although the exact mechanisms involved are not yet fully understood. Given that toxic effects of TCDD are closely related to its disposition, multiple physiologically based pharmacokinetic (PBPK) models of TCDD disposition, incorporating CYP1A2 induction, have been developed. In order to reduce uncertainties associated with extrapolation of these models across species, it is necessary that the mechanistic basis of TCDD sequestration in the liver be described as accurately as possible. To this effect, we are using PBPK modeling to investigate the mechanisms driving the hepatic dose response within a genetically diverse panel of mouse strains. We show that the differences in disposition across strains can be explained by a combination of AhR allelic categories, CYP1A2 induction and its binding affinity. Use of mechanistic insights from the mouse panel to refine PBPK modeling of TCDD will contribute to better understanding of TCDD induced hepatotoxicity and the reduction of uncertainty in risk assessment decision-making for dioxins.

Computational Modeling of an In Vitro Neural Network: Microelectrode Assay for Neurotoxicity Screening


Computational modeling of structural features of an in vitro assay can provide mechanistic insights into assay data. Here we describe computational modeling of an assay where primary rat cortical cells are grown over a microelectrode array for up to 12 days in vitro (DIV). As the neural network develops, random firing is detected at the electrodes by 5 DIV but, over time, firing becomes synchronized in bursts. The assay is used to screen for development of motor units, firing rates of excitatory and inhibitory neurons, fraction of all neurons in contact with electrodes, fraction of all neurons in contact with electrodes, and numbers of synapses formed by excitatory and inhibitory neurons. For example, when configured with 10n neurons, of which 75% are excitatory and 25% inhibitory, with 0.1% of neurons contacting electrodes, basal firing rates of 0.15 and 1.15 Hz for excitatory and inhibitory neurons respectively,
1822 Exploring Applications of Human Primary Cells for Drug Screening in Various Cell Culture Systems

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Human primary cells are useful pre-clinical models as they more closely mimic the physiology of cells in vivo as compared to continuous cell lines. Here we explored applications of human primary cells for cytotoxicity assays and drug screening. Cancer is a leading cause of female mortality worldwide and our ability to screen compounds against cancer is still lacking. Some of the common side effects of all anti-cancer drugs. We investigated the cytotoxic effects of three anti-cancer drugs, alone or in combination, on three types of normal reproductive cells in vitro. Primary human uterine fibroblasts, cervical epithelial cells, and vaginal epithelial cells were treated with topotecan, paclitaxel, cisplatin or a combination of these. Cells showed significant cytotoxicity. Topotecan and paclitaxel showed significant cyto toxicity in three types of reproductive cells at 0.1 µM while cisplatin significantly decreased the viability of all cells at 10 µM. Furthermore, the combination of topotecan and cisplatin treatment did not result in any significant additive effect on cytotoxicity of these reproductive cells. Three-dimensional (3D) cell culture systems utilizing primary cells may provide more physiologically relevant information and more predictive data in vivo assays. Skeletal muscle cells can contract under physiological conditions and their contraction may show damage during tissue injury and repair. To validate a 3D muscle contraction assay for drug screening applications, we embedded primary human skeletal muscle cells with a collagen matrix and treated them with 10 mM 2, 3-Butanediol 2-monoxime (BDM), a known inhibitor of skeletal and cardiac muscle contraction, for 19 days. The spontaneous contraction of untreated human skeletal muscle cells was observed in a time-dependent manner while BDM significantly inhibited contraction. The results from these assay systems demonstrate that various types of human primary cells can be used for pre-clinical applications including drug screening, toxicology, and modeling physiological responses.

1823 Using PBPK Modelling to Understand the Low-Dose Kinetics of Paraquat in Rodents and Dogs

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Paraquat dichloride (PQ) is a non-selective herbicide widely used in agriculture. The current work describes the quantitative low-dose kinetics of paraquat in rodents and dogs. A physiologically-based pharmacokinetic (PBPK) model of paraquat was developed using available data. It was also used to identify uncertainties and data gaps. New radiolabelled kinetic studies were conducted, and the PBPK model was revised as necessary. The starting point for creating the PBPK model was a minimal set of simplifying assumptions, namely no metabolism, linear kinetics with respect to dose, and clearance by urinary excretion of free plasma PQ at the glomerular filtration rate (GFR). Complexity was added only when necessary to simulate the available data. It was necessary to move from a flow-limited to a diffusion-limited model for each tissue allowing for tissue intertissue blood flow and tissue extracellular matrix and from tissue extracellular matrix to tissue intracellular matrix. It was also necessary to explicitly represent red blood cells, and to represent a slowly reversible component of plasma binding. Tissue rate constants were scaled between species according to tissue volume, and apart from this the PBPK model for each species was the same except for physiological parameters such as tissue volumes, blood flows and GFR. The current model now represents available mouse, rat and dog datasets very well for blood, plasma and tissue PQ concentration profiles and for clearance. This shows that the systemic behavior of PQ is the same in all these species, and differences in kinetic outcomes are driven only by physiological differences. This simplicity is a strength in that all available datasets are informing the parameterization of the same model, rather than studies on each species informing the parameterization of a species-specific model. This makes the resulting cross-species PQ PBPK model more robust. The dose routes represented are intravenous, subcutaneous, intraperitoneal, oral, dietary, and inhalation. The current model enables the estimation of blood, plasma and tissue PQ concentration profiles for any dosing regime, including for the many published PQ studies in which no kinetic measurements were made. The extension of the model to humans would help to inform risk assessments.

1824 Use of a PBPK-Quantitative Adverse Outcome Pathway Model to Resolve the Species Specificity of Lung Carcinogens


In mice, styrene, naphthalene, ethyl benzene, isoniazid and fluoro-sulfone form reactive metabolites via CYP2F2 in Club cells of the lung and produce lung-specific tumors. This association in humans is equivocal and considered to be species-specific for the mouse. Club cells (3.36 x 10^6 per mouse) comprise 80% of murine lung tissue but less than 8% of human lung. We propose that the mouse-human difference in carcinogenic potency is based on quantitative, rather than qualitative species differences. Our hypothesis is testable using PBPK models combined with a quantitative adverse outcome pathway (qAOP) linking formation of reactive metabolites with tumor development. Pathway progression in an AOP construct implies that a definable “response-response” (R–R) relationship exists between each key event (KE) leading to the adverse outcome (AO); Computational modeling of the R–R relationships enables dose-response evaluation of the probabilities and uncertainty, as well as cross taxa scaling and integration of new approach methodologies (NAMS; e.g. genomic or in vivo data streams). Using only published studies, we adapted a PBPK model for metabolism of styrene to styrene oxide and a qAOP implementation that includes a 2-stage (1) model with arms testing, Parallel models for different exposure scenarios: (1) direct metabolite damage of DNA, leading to errors of replication and increased mutation during cell division; and (2) indiscriminate metabolite damage resulting in cell death and regenerative proliferation without change to the background mutation rate per cell division. Chronic exposure drives progression to a mature lung cancer cell and club cell positive adenocarcinoma (the “A0); Preliminary findings indicate that the results of scale-up of the PBPK model from mouse to human using conventional allometric methods, incorporation of mouse-human differences in Club cell populations, and model calibration against relevant tumor data sets are consistent with qualitative species similarities and quantitative differences driving the mechanistic basis for the observed mouse-specificity of styrene as a lung carcinogen. This abstract does not necessarily represent the views or policies of the US EPA.

1825 Physiologically Based Pharmacokinetic Modeling the Impacts of Intermittent Oral Exposures to Lead on Blood Lead Levels and Associated Health Risks


Lead modeling has been used for many years to quantify the impact of lead intake on blood lead, bone lead, and other tissues in the body, in order to assess risks from lead exposure, as well as to set permissible levels for lead in environmental media. The International Commission on Radiological Protection (ICRP) model, also known as the Leggett model, is a well-regarded and validated physiologically based pharmacokinetic (PBPK) model for estimating blood lead levels resulting from oral or inhalation exposures. To investigate the potential health risks of various hypothetical lead exposure scenarios, we used the Leggett model to predict the quantitative impacts on blood lead associated with intermittent oral or inhalation exposure. Two case studies. First, we determined the geometric mean blood lead levels (averaged over a one-year exposure period) where the probability of exceeding blood lead level targets (5, 10, 15 or 20 µg/dL) is <=5% (assuming a geometric standard deviation of 1.8 for adults). We then determined the intake values (e.g. micrograms of lead per intake event) for a given exposure frequency (e.g. every other day, 1 day/week) that would correspond with those geometric mean values over a one year exposure period. In our second case study, we determined the exposure frequency (e.g. daily, once every seven days) for a hypothetical lead contaminated candy for a given lead intake (e.g. 3 µg lead per eating occasion) at which resulting blood lead levels fall below those associated with consumption of the California Office of Environmental Health and Hazard Assessment (OEHHA) Proposition 65 Safe Harbor level, or maximum allowable dose level (MADL) of 0.5 µg of lead every day. Based on our
evaluation of these hypothetical scenarios, we concluded that 1) daily point estimates associated with intermittent lead exposures are dependent on exposure frequency, 2) blood lead levels averaged over time are more dependent on total lead intake over a given time period than exposure frequency, and 3) modeling blood leads associated with intermittent exposures allows for evaluation of Proposition 65 compliance.

1826 Development of a Multi-route Physiologically Based Pharmacokinetic Model of Carbon Tetrachloride Uptake in Rats
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Carbon tetrachloride (CCl4) is a stable volatile compound; exposure results in dose-dependent hepatotoxicity. It is among the initial 10 chemicals prioritized for evaluation under the Lautenberg Chemical Safety Act. Due to its persistence in the atmosphere and environment, CCl4 has been detected in drinking water, creating multi-route exposure (inhalation, oral, dermal) exposure potential. While quantitative predictions for inhalation are well defined, quantitative predictions for oral exposure have large uncertainties, particularly for chronic reference dose calculations. To reduce uncertainty, physiologically-based pharmacokinetic (PBPK) models are used to predict internal dose metrics from external exposures. We used previously published pharmacokinetic data to build and calibrate a multi-route PBPK model for CCl4. Routes modeled include inhalation (100 and 1000 ppm) over 2 hours, single oral bolus administration (17.5 and 179 mg/kg), and intragastric infusion (17.5 and 179 mg/kg) over 2 hours. A two-phase GI absorption model was used for the oral exposures. This GI absorption model introduced four unknown parameters, each of which was fit or optimized. Local sensitivity analysis revealed that parameters to be identifiable, allowing calibration of the model and calculation of internal metrics. Predicted internal dose metrics, including maximum tissue concentration (Cmax), area-under-the-curve (AUC), and amount metabolized, were evaluated and correlated with experimentally observed liver toxicity. While the predicted amount metabolized did not correlate as well with toxicity (R2 = 0.607), liver Cmax and AUC did correlate (R2 = 0.9394 and 0.9254). Because metabolic activation of CCl4 is a requirement for hepatotoxicity, improved correlation between amount metabolized and toxicity would lead to greater model confidence and additional reduction in quantitative uncertainty in risk assessment. A logical next step is evaluation of the impact of oral absorption on model fit and predictions. This abstract does not reflect US EPA policy.

1827 Gaining Insights to Molecular Mechanisms of Combined Air Pollutants Exposures Using PBPK Models and Systems Biology Approaches
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Due to lack of accessibility, transparency and modeling knowledge, publicly available physiologically based pharmacokinetic (PBPK) models are rarely used in site-specific human health assessments. To address this issue, the Agency for Toxic Substances and Disease Registry (ATSDR) has developed a human PBPK model toolkit for multiple environmental priority pollutants using the Berkeley Madonna™ platform. The current work extends the ATSDR PBPK toolkit to include code models for additional volatile organic chemicals (VOCs) such as toluene, ethylbenzene, xylenes (TEX) and their combinations. Comparisons were made between simulation profiles and experimental data involving goodness of fits by calculating the percent median absolute performance error and root median square performance error. Results using the recorded and original models, as well as an application of the recorded models to evaluate VOC data are presented. Human gene expression changes in target tissues after short-term and long-term TEX mixtures exposure were assessed. This evaluation was then combined with toxicity enrichment analyses to examine VOC exposure related changes in disease-networks pathways. The combined analysis showed that 236 genes expressed were common between the short-term and long-term exposures. These genes are important for the interconnecting biological pathways potentially stimulated by TEX exposure, likely related to developmental or cardioprotective mechanisms. This work increases the accessibility of PBPK models, encourages their integration into chemical risk assessment, and allows the evolution of advanced methodologies that augment combined chemical dose-response analyses with mechanistic insights. 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.

1828 Neonatal Murine-Engineered Cardiac Tissue Toxicology Model: Impact of Metallothionein Overexpression, Cadmium Exposure, and Zinc Countermeasures
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Engineered cardiac tissues (ECT) are robust in vitro models to study human disease. Environmental exposure to heavy metals is a global health risk and metallothionein (MT) is cardioprotective for environmental toxicants such as the heavy metal, Cadmium (Cd). Zinc (Zn) supplementation increases MT along with reduction of Cd metal toxicity. First, therefore, we generated 3D ECTs from PN day 3 wild-type (WT) or cardiomyocyte (CM) MT- transgenic (MT-TG) mouse cardiac cells to test the hypothesis that ECT maturation and function would not be adversely affected by MT-TG CM genotype. We found that structural and functional maturation was similar in WT and MT-TG neonatal ECTs. MT gene and protein expression was similar between MT-TG ECT and MT-TG whole ventricle. We then tested the hypothesis that Cd exposure would adversely affect murine ECT function and CM viability, similar to animal model observations, and that zinc or Zn pretreatment could reduce Cd mediated MT overexpression of MT would reduce Cd mediated CM toxicity in ECT. Starting on day 6, WT and MT-TG ECTs were treated with Cd (20-50 μmol/L) for 24 hr. Additional WT ECTs were pre-treated with Zn (50 μmol/L) on day 5 followed by Cd treatment on day 6. We found Cd exposure negatively impacted WT ECT cell survival, maturation, and function quantified by reduced ECT function and increased cleaved caspase 3, Bax/Bcl2 ratio, TUNEL positive cells consistent with CM loss. All these effects were significantly prevented in MT-TG ECTs and in Zn-pretreated WT ECTs with MT overexpression. Thus, neonatal murine ECTs can serve as a robust in vitro model for CM toxicity screening and as a platform to evaluate the role of cardioprotective mechanisms such as MT-overexpression, and metal toxicity countermeasures, such as Zn, relevant to human heavy metal environmental exposure.

1829 Evaluating the Impact of Heat Stress on the Toxicokinetics of Volatile Solvents in Man Using PBPK Modeling
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In the context of climate change, there is a growing concern about how temperature might affect absorption of chemicals. To address this question, volunteers were exposed in an inhalation chamber at three different temperatures with and without solvents. Blood, urine and exhaled air samples were collected before, during and after 4h inhalation exposures. Respiratory parameters were also monitored for each temperature. No significant changes were observed in exhaled air or urine samples for inhalation exposures and no changes were observed for blood concentrations following toluene cutaneous exposures. Results showed increased blood concentrations (over 20%) at the end of the 4h exposure periods for the highest temperature for all solvents with and without solvents. Blood, urine and exhaled air samples were collected before, during and after 4h inhalation exposures. Respiratory parameters were also monitored for each temperature. No significant changes were observed in exhaled air or urine samples for inhalation exposures and no changes were observed for blood concentrations following toluene cutaneous exposures. Results showed increased blood concentrations (over 20%) at the end of the 4h exposure periods for the highest temperature for all solvents with and without solvents. Blood, urine and exhaled air samples were collected before, during and after 4h inhalation exposures. Respiratory parameters were also monitored for each temperature. No significant changes were observed in exhaled air or urine samples for inhalation exposures and no changes were observed for blood concentrations following toluene cutaneous exposures. Results showed increased blood concentrations (over 20%) at the end of the 4h exposure periods for the highest temperature for all solvents with and without solvents. Blood, urine and exhaled air samples were collected before, during and after 4h inhalation exposures. Respiratory parameters were also monitored for each temperature. No significant changes were observed in exhaled air or urine samples for inhalation exposures and no changes were observed for blood concentrations following toluene cutaneous exposures. Results showed increased blood concentrations (over 20%) at the end of the 4h exposure periods for the highest temperature for all solvents with and without solvents.
Physiologically Based Pharmacokinetic Modeling to Estimate Bone Marrow Doses of Hydroquinone from Smoking

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Chronic benzene exposure is associated with increased risk of acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). Cigarette smoking constitutes a large contribution to personal benzene exposure; smoking is also reported to be an environmental risk factor for AML and MDS. Benzene is one of many bone marrow toxicants present in cigarette smoke; however, smoke also contains hydroquinone (HQ), which is reportedly the most abundant oxidative compound present in cigarette smoke and is potentially the most potent benzene metabolite contributing to the myelotoxicity of benzene. PBPK models were used to estimate the amount and concentration of HQ and total metabolites in blood and bone marrow resulting from inhalation exposures to both benzene and HQ from smoking exposures (20 or 40 cigarettes per day). An existing PBPK model for benzene was used to estimate the HQ internal dose resulting from metabolism of benzene; the benzene PBPK model was then modified to simulate direct inhalation exposure to HQ from cigarettes. Inhaled concentrations of benzene and HQ from cigarette smoke were estimated to be nearly equivalent. Assuming that 20 or 40 cigarettes are smoked over the course of a 16 hr waking period during the day and that each cigarette smoking event lasts 10 minutes, steady-state concentrations of benzene and HQ were reached in blood and bone marrow in approximately 10 days. Steady-state blood and bone marrow concentrations and cumulative amounts of HQ resulting from direct inhalation of HQ from cigarettes were 5- to 27-fold higher than bone marrow and blood concentrations of HQ resulting from benzene inhalation alone from cigarettes. The sum of HQ and total metabolites in bone marrow and blood were 3- to 29-fold higher than from benzene exposure in cigarettes alone. This model was used to estimate the inhaled concentration of HQ in bone marrow as benzene; this inhaled HQ concentration was 5 times lower than the benzene concentration. This study demonstrates that HQ in cigarette smoke results in much higher blood and bone marrow concentrations than the presence of benzene alone at nearly equivalent inhalation concentrations of HQ and benzene. HQ should also be quantified in assessing potential MDS or AML risks from chronic smoking.

Assessing Early Life Drinking Water Exposures to Manganese (Mn) Using PBPK Modeling

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A previously published human PBPK model for Mn in infants and children, which was able to successfully capture age-dependent Mn homeostasis at diurnal steady state, was updated to reflect recent information on Mn metabolism. Various Mn exposure scenarios, which fall within the observed variation of Mn concentrations in infants' blood in Stastny et al. study. This multi-route, multi-source Mn PBPK model for infants and children will contribute to evaluating the realistic impact of drinking water exposure to Mn in early age populations and will thereby improve the level of confidence in understanding exposure-health effects relationships reported to be associated with early age Mn exposure in human epidemiological studies.

Psensi: An R Package to Apply Sensitivity Analysis in Pharmacokinetic Modeling

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Sensitivity analysis is an essential tool for modelers to understand the influence of model parameters on model outputs. It is also increasingly used in developing and assessing pharmacokinetic models. In our previous work, we applied a global sensitivity analysis workflow to reduce the computational burden in the Bayesian Markov Chain Monte Carlo-based calibration process of a physiologically based pharmacokinetic (PBPK) model. Although several sensitivity analysis algorithms are available, comprehensive package exists that allows users to seamlessly solve the PBPK model differential equations, run sensitivity analyses, visualize sensitivity analysis results, and discriminate between the “non-influential” model parameters that can be fixed and those that need calibration. Therefore, we developed an open-source R package, called psensi (https://github.com/nanhung/psensi), to fill this need and make sensitivity analysis more accessible in pharmacological and toxicological research. This package can investigate both parameter uncertainty and model formulating sensitivity analysis and can create robust and reproducible results for
Population physiologically-based pharmacokinetic (pop-PBPK) models are powerful tools in toxicology, pharmacology, and regulatory science. One of the critical challenges in population PBPK modeling is the identification and estimation of critical model parameters at desired levels of an analysis hierarchy, from individual subject, to group, to population. Although other methods have been used for this parameterization, a hierarchical Bayesian approach is particularly well suited for this application. Unfortunately, the adoption of Bayesian pop-PBPK modeling has been hampered by a number of factors, including difficulties in identifying influential model parameters, developing the appropriate structural and statistical models, specifying the relevant parameter priors and Bayesian likelihood distributions, and finding a simulation framework to conduct the relevant analyses. To fill this important gap, we developed PoPKAT, a user-friendly, open-source platform that facilitates pop-PBPK analyses that includes state-of-the-art Bayesian sampling algorithms, parameter space reduction through global sensitivity analyses, and a graphical user interface for convenient entry of simulation information and viewing and interpretation of simulation results. Here, we detail the structure and computational capabilities of the framework and illustrate its utility for the analysis of the pharmacokinetics of several compounds of toxicological and pharmacological relevance across structural hierarchies of interest.

Heat stress is of significant concern to Department of Defense personnel in extreme environments. The human body preserves an internal temperature of ~37°C to maintain a healthy metabolism and employs several homeostatic mechanisms such as blood vessel dilatation/constriction and decreased sweating to control this temperature. These mechanisms may disrupt normal toxicokinetic activity within the body. Concurrent heat stress and toxic chemical exposure can be a concern in the DoD occupational environment. Our objective was to provide individual analyses of the thermal and chemical exposure risks to assess the potential effects of these exposures in the extreme environments. Human thermal and chemical exposure data were gathered for ~30 individuals. The data were gathered using direct-reading, real-time gas monitors in the breathing zone for carbon dioxide, carbon monoxide, nitrogen dioxide, nitric oxide, and hydrogen sulfide. Thermal desorption tubes were also used in conjunction with sampling pumps to determine the presence of other gases and vapors. The thermal desorption tubes were analyzed off-line using gas chromatography-mass spectrometry. Temperature data were gathered via HOBO® temperature data loggers to gather local air temperature and Zephyr™满怀电流 devices to gather heart rate, respiration rate, and skin temperature to estimate core body temperature. A physiologically based pharmacokinetic model was built that incorporated a thermal stress model. The model was validated using data gathered from human venous blood concentrations after being exposed to 50 ppm toluene at three temperatures within the body. Concurrent heat stress and toxic chemical exposure can be a concern in extreme environments. Heat stress is of significant concern to Department of Defense personnel.

Mitochondria are the important organelles to maintain the cellular bioenergetic balance and are involved in the progression of adversity in many target organ toxicities. Various enzymes involved in fatty acid oxidation and the citric acid cycle as well as the mitochondrial respiratory chain can be target of chemical interference. Adaptation of mitochondria to perturbations is critical to modulate the balance between recovery from mitochondrial stress aiming at cell survival, and energetic collapse leading to onset of intrinsic apoptosis or necrosis. Here we aimed to obtain a deeper understanding on mitochondrial adaptation upon exposure to various mitochondrial respiration inhibitors by combining modeling, machine learning and high-content imaging that quantitatively captures different mitochondrial malfunctioning, e.g. mitochondrial membrane potential (MMP) and mitochondrial morphology. We treated HepG2 cells with a set of >20 mitotoxicants that inhibit complex I, II or III, the mitochondrial F$_{0}$F$_{1}$ATPase or act as uncoupler. First, to represent the respiration chain, we adapted an existing differential equation model describing the MMP and cellular ATP levels. We optimized the values of the model parameters to fit our imaging data monitoring the MMP (Rho123) dynamics for 24 hours. We extended the model with MMP leakiness in order to obtain an acceptable fit to our time-concentration response data. Second, since some of the mitotoxicants disturbed mitochondrial morphology, we aimed to integrate dynamic mitochondrial morphology changes in the model. In order to quantify these effects, we applied a random forest approach to segment mitochondria from our images. We subsequently exploited features from the segmented mitochondria such as form factor and area by applying a Gaussian mixture model to classify mitochondria as either fragmented or fused. We established a preliminary differential equation model that could qualitatively mimic the observed amounts of fragmented/fused mitochondria upon compound exposure. We aim to integrate all above sub-models into one holistic model. We expect our data-driven computational approach to provide an improved mechanistic and comprehensive understanding of the sequence of events leading to mitochondrial toxicity and consequently cell.
1838 Extending the PLETHEM Platform for PBPK Modeling: Batch Mode Processing, Dermal Route of Exposure, and Integration with Mode-of-Action Tools


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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 estimates to physiologically based pharmacokinetic (PBPK) modeling and computational systems biology pathway modeling tools into a standardized framework. Over the last year, we implemented support for exposure estimation tools under our PLETHEM framework. We now have further extended the modeling capabilities in PLETHEM by adding a batch mode for running multiple chemicals at once and incorporating a dermal route of exposure. Using HT-IVIVE workflows within PLETHEM we can now extrapolate in vitro to an in vivo dataset of chemicals and establish a margin of exposure using exposure estimation tools. Integrating support for MoAviz, exposure estimation tools and multiple routes of exposure will meet the need for a unified multi-route tool that can be used along the source-to-outcome continuum. This research is funded by the ACC-LRI and is conducted under an MoU with US EPA NCTR.

1839 Evaluations of the Utility of HTTK-PBT model for In Vitro to In Vivo extrapolation

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Physiologically based toxicokinetic (PBTK) models allow for extrapolating in vitro data to predict the overall in vivo pharmacokinetics of drugs or chemicals (IVIVE). The High-Throughput Toxicokinetic (HTTK) tool, built as an R package, can be used to efficiently evaluate a large number of compounds using PBTK modeling. The derived set of compounds used to establish a mode of action can be directly imported into PLETHEM. The HT-IVIVE workflow can then be used to extrapolate these PODs to exposures and establish a margin of exposure using exposure estimation tools. Integrating support for MoAviz, exposure estimation tools and multiple routes of exposure will meet the need for a unified multi-route tool that can be used along the source-to-outcome continuum. This research is funded by the ACC-LRI and is conducted under an MoU with US EPA NCTR.

1840 Use of Risk Assessment for Innovative Reuse of Dredged Sediment


Sponsor: M. Ciarlo, Society of Environmental Toxicology and Chemistry

The Port of Baltimore is an economic driver for the State of Maryland. The Port contributed $3 billion in gross revenue, $300 million in state and local tax revenues, and provides over 100,000 jobs. The maintenance dredging of the federal navigation channels that lead to the Port is imperative to sustaining this economic driver. For the Maryland Port Authority (MPA), the management of dredged material from federal navigation channels is a major concern. On average, 5 million cubic yards (mcy) of dredge material is removed annually from the Potomac River and delivered to the Maryland Port and Clipper City Dredge Material Reclamation Area in the Chesapeake Bay. Of this total, 1.5 mcy is removed from the Baltimore Harbor. Material removed from the Baltimore Harbor is currently placed in one of two dredged material containment facilities as prescribed in Maryland State Law. Due to limited available land along the Baltimore Harbor, it is becoming increasingly infeasible to construct additional containment facilities to meet the future needs of the MPA. To account for the infeasibility of constructing additional facilities, the MPA has identified the re-use of dredged material as a primary mechanism to meet future needs. The MPA has a goal to re-use 500,000 cy of dredged material per year with on land or upland placement as the most feasible alternative to meet the re-use goal. Before 2017, there was no guidance or program the MPA could follow for the re-use of this material. EA Engineering, Science, and Technology (EA) assisted the MPA, in conjunction with the Maryland Department of the Environment (MDE), to develop a new guidance to facilitate the re-use of dredged material. The guidance uses risk assessment to determine risk-based screening criteria for sediment. The guidance also provides steps to determine if the material is acceptable for re-use at a variety of sites. To account for the wide range of chemical concentrations in the dredged material, the screening criteria evaluates various land uses. Additionally, the guidance allows for the use of the risk assessment process to further evaluate materials that exceed screening criteria but still may be available for re-use by taking into account the location and anticipated land use where dredged material will be placed. By evaluating potential current contamination at the location, planned use or redevelopment of the site, material that previously had to be disposed in a containment facility has the potential to be re-used at sites. Additionally, the risk assessment process ensures reduction in future liability of the MPA for placement of material at the sites by providing a quantitative determination of potential risks based upon anticipated site use.

1841 Improving Read-Across Assessment of Cosmetic Ingredients Utilizing COSMOS DB In Vivo Data and In Silico Evidence to Reduce Uncertainties


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Read-across is a well-established technique utilizing data-rich substances to fill gaps for data-poor compounds. It is particularly useful for the assessment of toxicological endpoints for cosmetic ingredients. In order for read-across predictions to be acceptable, key uncertainties must be established and, where possible, areas of high uncertainty must be identified. We demonstrate how in silico tools can be used for risk assessment and increasing confidence in the read-across predictions. This study identified analogues for the data-rich substance maltol. Toxicity data for maltol were obtained from COSMOS DB (cospodb.eu). In addition to a NOEL value from a 180-day mouse oral study, a toxicity data package is available across endpoints including acute, genetic and reproductive toxicities as well as carcinogenicity and organ level effects across a variety of species and exposure routes. The available data were found to be of high quality; as such, maltol is an ideal data-rich “source compound” for read-across. In silico tools within the updated COSMOS DB were utilized to identify “target compounds” based on structural and molecular properties. Structural similarity was assessed using various types of fingerprints (e.g. ToxPrints). Property-based similarity was addressed by calculating sets of relevant physicochemical and electronic descriptors for source and target compounds. In silico screening was performed to reduce uncertainties related to similarity and mechanistic plausibility. The newly implemented and freely available Liver BioPath profiler in the COSMOS DB was applied to characterise source and target compounds using structural knowledge on reactive hepatotoxicity, phospholipidosis, steatosis, mitochondrial effects and other general toxicities to the liver, the most important target
arsenic inorganic arsenic in the 100s to 1000s of ug/L (ppb) has been found to increase the risk of a variety of cancers (Lung, bladder, skin, and possibly prostate). While a review of the literature on arsenic and lung cancer (Lamm et al., 2015) showed a good fit to a linear-quadratic model with a significantly negative linear term and a significantly positive quadratic term, most analyses have reported models with only a significantly positive linear term. We examine here the risk of prostate cancer using recent US county incidence data and ground water well arsenic levels up to nearly 1,000 ug/L arsenic. Groundwater well usage and arsenic levels were obtained from the US Geological Survey, prostate cancer incidence rates from the National Cancer Institute, and co-variate data from the US Centers for Disease Control and Prevention and the US Bureau of the Census. All data were aggregated at the County (FIPS) level and analyzed with Poisson regression analysis. A total of 738 counties (FIPS code areas) had arsenic measurements for their drinking water supply and had prostate cancer incidence data. In analyses conducted for areas with median arsenic level less than 60 ug/L, the risk of prostate cancer risk was found to fit a linear-quadratic model with a significantly negative linear coefficient and a significantly positive quadratic coefficient. In analyses limited to areas with median arsenic level less than 30 ug/L, the risk of prostate cancer risk was found to fit a linear model with a significantly negative linear coefficient. The addition of a quadratic term did not significantly improve the fit of the model. Prostate cancer incidence risk with respect to arsenic levels in drinking water sources for US counties (FIPS codes) did not fit a linear model with a significantly positive quadratic coefficient. The most important factor for establishing a screening value. The resulting derivation HBSS for THC on surfaces was 0.7 µg/100 cm^2. Paired samples taken before and after cleaning surfaces from naïve (vacant following police raid, eviction, etc.; uncleaned or minimally cleaned) clandestine grow operations in Washington State residences show that (1) surfaces can exceed the derived screening level and (2) concentrations on surfaces are effectively reduced following cleaning. Use of the derived screening value will allow stakeholders to evaluate areas that may require remediation and can be used to assess the effectiveness of subsequent cleanup efforts.

Increased risks have been observed for multiple health outcomes in populations exposed to inorganic arsenic in water and diet. Comparing exposure/dose-response is complicated because varying dose metrics (water arsenic concentration, dietary arsenic intake, urinary arsenic) have been used to analyze exposure-response. In addition, relatively simple, monotonic functional model forms have often been used, making it difficult to discern curve shape, particularly at low exposures. This presentation describes a meta-regression approach undertaken to investigate the shape of exposure/dose-response for bladder cancer, a well-studied health outcome associated with arsenic exposure. Exposure-response data for arsenic health effects was obtained from high-quality epidemiological studies identified in a Systematic Literature Review conducted by the US EPA. The selected studies report results from populations in the U.S., Japan, and Taiwan with a broad range of exposures, including considerable data at or only slightly above U.S. background levels of exposure. A unique feature of the approach is that the reported exposure, dose, or excretion metrics were all converted to chronic daily intake using data from a peer-reviewed PBPK model, enabling studies to be compared on a common basis. In addition, pooled dose-response relationships were estimated using fractional polynomial (FP) mixed models. The FP methodology systematically investigates second-order functions that accommodate a very wide variety of non-monotonic curve shapes, including those with negative slope near the origin (horneric or “hook-shaped”). Best-fitting models for the pooled data were identified based on AIC, deviance, and R^2^adj values. Generally, MLE predictions from the best models were convex upward with positive slope at low doses, although resolving model behavior at low doses was difficult owing to high inter-study heterogeneity. Leave-one-out and group-wise analyses were used to identify studies with the most impact on the pooled dose-response estimates. Disclaimer: The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the US EPA.
2-Nitropropane (2-NP) is a solvent in inks, dyes, and adhesives as well as an additive in propellants and racing car fuels. It was listed on the State of California’s Proposition 65 list in 1988 reflecting carcinogenic potential based on tumorigenicity in rats. California does not list a Safe Harbor Level for 2-NP. In this analysis, oral and inhalation No Significant Risk Levels (NSRLs) were derived for 2-NP. Additionally, an exposure assessment was performed to determine potential consumer inhalation exposure to 2-NP during indoor application of spray paint to verify that proposed NSRL was not exceeded. Benchmark Dose modeling (BMD) was conducted using combined hepatocellular carcinoma incidence data from several single exposure inhalation studies in Sprague-Dawley rats. A sensitivity analysis was conducted to evaluate the impact of various time averaging assumptions and use of alternative and averaged BMDL values. The model averaged BMDL for male rat hepatocellular carcinoma incidence was used as the point of departure to derive the inhalation human cancer potency based on the ratio of the uptake factors for the inhalation versus the oral routes. The inhalation and oral NSRLs were determined to be 60 μg/day and 32 μg/day, respectively. The estimated exposure to 2-NP for the spray paint scenario was found to be a small fraction of the proposed NSRLs. Based on the screening exposure assessment, potential consumer exposure to 2-NP from indoor application of spray paint does not exceed the proposed NSRL, and a warning label would not be required for spray paint products.

**1847 Risk Characterization of Bisphenol-A in the Slovenian Population Starting from Human Biomonitoring Data**

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The current study aims to characterize exposure and risk associated to bisphenol-A (BPA) exposure in Slovenia, starting from biomonitoring data. Based on urinary data, individual daily intake was back-calculated using a generic physiology-based biokinetic (PBBK) model parameterized for BPA and coupled with an exposure reconstruction algorithm. Re-running the PBBK model in forward mode allowed the estimation of biologically effective dose (free plasma BPA) and the respective daily area under the curve (AUC). The risk characterization ratio was derived using both external and internal dose metrics. Urinary BPA levels were found low, with GM of 0.79, 1.51 and 0.20 μg/g creatinine for mothers, children and fathers respectively, similar to the levels of other European countries. Based on the above and accounting for the dynamics of exposure and biokinetics, the daily intake was estimated with median values equal to 0.019, 0.035 and 0.005 μg/kg bw/d for mothers, fathers and children respectively. The highest estimated intake level was found in a child, equal to 0.87 μg/kg bw/d, while the maximum intake for mothers and fathers were 0.7 and 0.8 μg/kg bw/d respectively. The respective RCR values using the EFSA t-TDI of 4 μg/kg bw/d were 2 magnitudes of order lower than unity, independently of the selected method. Had daily intake been estimated solely based on the urinary concentration mass balance, the estimated intake would be lower, due to the assumed simplifications in exposure and elimination time dynamics. Our results highlight the importance of using PBBK modelling-based exposure reconstruction schemes for rapidly metabolized and excreted compounds such as BPA. In this context, deriving the optimal sampling scheme (number of timing of samples within the day) is crucial for risk characterization in the case of rapidly metabolized compounds.

**1848 High-Fat Diet Promotes the Obesity Induced by Perinatal Exposure to 4-Nonylphenol in Rats**

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Human beings are constantly exposed to more than one toxicant; therefore, it is essential to elucidate the mechanism of combined effect from toxicants. Herein we determined the effect of perinatal exposure to rats with 4-nonylphenol (4-NP) and high-fat diet (HFD) in the adipogenesis of F1 and F2 generations. We fed F0 female rats with 4-NP by gavage. Then the F1 generation rats were divided into 4 groups: control group, fed with normal diet (ND); NP group with 4-NP plus ND; HFD group, with HFD; and NP/HFD group, with 4-NP and HFD. Male rats from control group were mated with female rats from NP group and NP/HFD group to get the F2 rats. After weaning, F2 offspring rats were given normal diet. Compared with control group, the 4-NP and HFD have synergistic action on the organ coefficient of adipose tissue, blood biochemical indexes, the mechanism of gene related from Cdc25 synthesis in F1 and F2 rats. Both HFD and 4-NP can also play a synergistic role in the reduction of estrogen receptor in adipose tissue of F2 rats. We conclude that HFD fed to the F1 generation rats can aggravate the 4-NP effect on the adipose tissue formation of F2 generation rats.

**1849 Chemical Reactivity, Not Molecular Weight or Lipophilicity, Determines Skin Sensitization Potential and Potency: An Analysis Based on the Latest Public Data for Set of Chemical Substances Tested for Skin Sensitization**

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Skin sensitization is induced when a susceptible individual is exposed topically to haptens. This chemical sensitizer (hepten) provokes a cutaneous immune response that will subsequently result in the development of contact sensitization. For many years, it has been widely accepted that substances must have a molecular weight (MW) of less than 500 to penetrate effectively through the skin to induce sensitization. We have analyzed the latest public data set of approximately 5000 chemical substances tested for skin sensitization to further understand the physico-chemical characteristics of chemical substances that are the key determinant(s) for inducing skin sensitization. The MWs of sensitizing substances were in the range of 30 to 2300, excluding high MW proteins. The high sensitization potency has been shown with several high MW substances (2300 > MW > 500) with low dermal absorption. The measured values of octanol/water partition coefficient (Kow) for sensitizing chemical substances have been found as low as 10⁻¹ and as high as 10³, thus encompassing a range of ten orders of magnitude. There was no correlation between sensitization potency and MW (or Kow). The mere presence of a reactive functional chemical group(s) within a chemical substance did not automatically suggest that the respective substances are highly potent sensitizers. Nevertheless, our analysis of the latest public data set of chemical substances tested for skin sensitization suggests that: (1) the key determinant for skin sensitization is the chemical reactivity of a given substance; a property that is inherently protein-reactive or that can be metabolized in the skin to reactive species; and (2) as long as a chemically reactive substance can gain access to the viable epidermis, it can induce sensitization or exhibit high sensitization potency regardless of its ability to penetrate effectively through the skin.

**1850 Analysis of Environmental Chemical Exposure of the Canadian Population from the Canadian Health Measures Survey in a Risk Based Context**

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In order to characterize the exposure of the Canadian population to environmental chemicals, a human biomonitoring component has been included in the Canadian Health Measures Survey (CHMS). This nationally representative survey launched in 2007 by the government of Canada has measured over 250 chemicals in approximately 30,000 Canadians during the last decade.
The capacity to interpret these data in a health risk based context is gradually improving with the development of biomonitoring screening values, such as biomonitoring equivalents (BE) and human biomonitoring (HBM) values. This study aims to analyze recent CHMS biomonitoring data in a health risk based context in order to assess the exposure of the Canadian population and help prioritize chemicals. Hazard quotients (HQs) are used in this analysis and are calculated as the ratio of the CHMS biomonitoring data to the corresponding biomonitoring screening value for individual environmental chemicals at both the geometric mean (GM) and 95th percentile (PP95). This analysis includes short half-life chemicals, persistent chemicals, and volatile organic compounds (VOCs). Most of the chemicals analyzed have HQs below 1 suggesting that exposure to those compounds is not currently of concern. However, HQs exceed 1 in smokers for cadmium, acrylamide, benzene and xylene, as well as in general population for inorganic arsenic and fluoride. Specifically, for inorganic arsenic in the general population an HQ value of 3.13 was calculated at the 95th percentile. For fluoride, HQs were 1.23 and 1.67 at the 95th percentile, respectively. This study suggests that exposure of the Canadian population to these environmental chemicals might be a concern. This screening exercise is useful to help prioritize chemicals for follow-up activities such as risk management actions and show the importance of a continued biomonitoring program to assess population exposures.

1851 Development of Inhalation Reference Concentrations for Chlorotrifluoroethylene (CTFE) and 1,2-Dichloro-1,2,2-trifluoroethane (HCFC-123a)

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The presence of CTFE and HCFC-123a in the subsurface at a site targeted for residential development led to a need for key toxicological data to derive toxicological reference values for human health risk assessment. A literature search revealed that CTFE produces renal toxicity by formation of glutathione S-conjugated metabolite(s) in the liver, which are then hydrolyzed enzymatically to cysteine S-conjugates and reactive intermediates in renal tubules causing dose-dependent renal toxicity. The lowest concentration (29 ppm) in a subchronic inhalation toxicity study was identified as a minimal LOAEC based on statistically significant increases in serum albumin and absolute and relative liver and kidney weights. Converting the minimal LOAEC to a human equivalent concentration (HEC) using a default animal:human ratio of 1 for the blood:gas partition coefficient for extrarespiratory effects, adjusting the HEC to continuous exposures, and applying a composite uncertainty factor of 3,000 (3 for UFHEC, 10 for UF10, 10 for UF20, 3 for UF3, and 3 for UF) yielded a reference concentration (RfC) of 8 µg/m³ for CTFE. Due to the absence of toxicological data for HCFC-123a, a surrogate approach was used to estimate a RfC using data for HCFC-123. HCFC-123 and HCFC-123a share the same molecular formula but differ in the location of the fluorine and chlorine atoms on the carbon backbone. HCFC-123 and HCFC-123a, are converted to trihaloacetic acids through a metabolic pathway that produces reactive trihaloacetyl intermediates which are believed responsible for the toxicity of the parent compound. Toxicokinetic data available for HCFC-123 included occupationally, acute, subchronic, teratological, one and two generation reproductive, and two-year chronic studies. A surrogate LOAEC of 300 ppm for HCFC-123a, based on decreased body weight and body weight gain and significant changes in clinical chemistry parameters from a chronic inhalation bioassay with HCFC-123, was identified as the most appropriate point of departure. Converting the LOAEC to a HEC using a default animal:human ratio of 1 for the blood:gas partition coefficient for extrarespiratory effects, adjusting the HEC to continuous exposures, and applying a composite uncertainty factor of 10,000 (10 for UFHEC, 10 for UF10, 10 for UF20, 3 for UF3, and 10 for UF) yielded a preliminary RfC for HCFC-123a of 34 µg/m³. The conservative RfCs were reviewed and accepted by state regulatory officials.

1852 Derivation of a No-Significant-Risk Level (NSRL) for Pyridine

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Pyridine, a commonly used organic solvent and chemical synthesis intermediate, has been listed on the State of California’s Proposition 65 List as a chemical known to the State to cause cancer. This listing is based on the results of a National Toxicology Program (NTP) two-year drinking water carcinogenicity study in rodents which identified clear evidence of carcinogenic activity in B6C3F1 mice based on increased incidences of liver neoplasms in both sexes, and evidence of carcinogenic activity in female CD rats based on increased incidences of renal neoplasms in males and mononuclear cell leukemia in females. Industries are obligated to comply with the Proposition 65 labeling requirement and drinking water discharge prohibition for products sold in the State of California, unless they are able to demonstrate that pyridine levels in their products result in consumer exposures that are below a specific safe harbor level, known as a No Significant Risk Level (NSRL) for carcinogens. The State of California has to date not published a NSRL for pyridine. In accordance with California EPA guidelines, ToxServices derived a NSRL for carcinogenic activity of 10 µg/L obtained from the NTP carcinogenicity study using benchmark dose modeling. The US EPA’s Benchmark Dose Software (BMDS) was used to model the cancer slope factors, a measure of the risk of carcinogenesis per unit dose, for the single and combined incidences of hepatic neoplasms in male and female mice, single and combined incidences of renal neoplasms in male rats, and incidence of mononuclear cell leukemia in female rats. When inadequate goodness of fit was obtained (p < 0.1), ToxServices dropped the highest dose group from the benchmark dose (BMD) modeling and then performed the modeling on the smaller dataset to determine if the goodness of fit improved. The slope factors derived from BMD were scaled to human equivalent doses (HEDs) using body weight scaling, and the resulting HEDs were used to determine the lifetime average daily pyridine dose that would result in one excess cancer case in an exposed human population of 100,000 (10⁵ cancer risk). The most conservative and health-protective NSRL of 3.9 µg/day was identified from the increased incidence of hepatocellular carcinoma in male mice following pyridine exposure in drinking water. This NSRL can be used as part of an exposure and safety assessment to evaluate compliance with Proposition 65 requirements for consumer products containing pyridine and sold in the State of California.

1853 Derivation of a Screening-Level No Significant Risk Level (NSRL) for Tris(2-Chloroethyl) Phosphate (TRCP)

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TRCP, a flame-retardant and plasticizer used in flexible and rigid foams, is included in California’s Proposition 65 list of chemicals known to the State to cause cancer or reproductive toxicity because of its potential carcinogenicity. This poster derives a screening-level No Significant Risk Level (NSRL) for TRCP. For carcinogens or potential carcinogens, a NSRL is equivalent to an exposure level that results in 1 excess cancer in an exposed human population of 100,000, assuming lifetime exposure at the level in question (27 CCR §25703). Clear evidence for renal tubule cell adenomas and carcinomas was exhibited in a 104-week oral toxicity study with male F344/N rats (NTP 1991) as well as an 18-month oral toxicity study with male ddY mice (Takada et al. 1989). As part of a provisional peer reviewed toxicity value report (PPRTV), the US EPA used benchmark dose modeling to derive a 0.02 (mg/kg-day) cancer slope factor (CSF) for renal tubular cell adenomas and carcinomas in male F344/N rats (US EPA 2009). A literature search confirmed that no additional cancer studies have been published since derivation of the CSF. Incorporating US EPA’s CSF into a calculation to obtain the dose associated with 1 in 100,000 cancer risk while assuming lifetime exposure results in a NSRL of 35 µg/day TRCP. This NSRL value can be used for Proposition 65 compliance determination and evaluation of health risks.

1854 Development of a QSAR Model to Predict Respiratory Irritation by Individual Constituents

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The RespiraTox project, funded by NC3R CrackIT, develops a QSAR model, which predicts the potential of individual compounds to cause irritation in the respiratory tract. We distinguished two mode of actions, i) "sensory irritation", characterized by a decrease in breathing rates, and ii) "tissue irritation" characterized by primarily histopathological findings. QSAR models rely on high quality datasets. We based the classification "irritating to respiratory tract" on several data types from in vivo studies with inhalation exposure. In a tiered approach, we considered information from i) studies with acute exposure from the ECHA CHEM database (DB), ii) the Hazardous Substance DB, iii) the harmonized classification and labelling inventory from ECHA, and iv) repeated dose studies from the Fraunhofer RepDose DB. For later stage validation, we withheld human data from Fraunhofer Breath DB. The final data set includes about 2500 irritating and 800 non-irritating compounds. Prior to model development, the CAS numbers and compound structures were quality controlled and corrected. Two kinds of information were generated
from the compounds structures: i] structural descriptors (ECFPs), and ii] physico-chemical properties. We explored several machine learning algorithms including Logistic Regression (LR), Random Forests (RF), and Gradient Boosted Decision Trees (BT) to derive a classification model. The internal validation procedure employed stratified k-fold cross-validation (k=5). The overall approach adheres to the five OECD principles. The criteria used to measure performance of the model is chosen by receiver operating characteristic (ROC) curve (AUC). The AUC for LR using the combined feature set is 0.71. The optimal performance for both RF and BT is 0.78. The applicability domain is determined by features with highest impact on the final model. The current approach will be further refined and improved (e.g. by differentiating sensory and tissue irritation). The final model will be provided online as a friendly interface to be used by toxicologists, regulators, and overall to reduce the testing of animals.

1855 Updated Modeling Indicates Historical Sterilization Worker Ethylene Oxide Exposures Were Higher Than Assumed in the US EPA IRIS Assessment

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The 2016 US EPA IRIS assessment cancer slope of 5 x 10^{-3} (µg/m^3)^{-1} for ethylene oxide (EO), which implies that a chronic inhalation of just 0.1 parts per trillion of EO in air has a 10^{-4} increased cancer risk, was based on NIOSH epide- miology studies of workers exposed to EO in contract sterilization operations between 1938 and 1986. That assessment relied on exposure estimates NIOSH developed for those workers using a statistical regression model validated by fitting EO air-monitoring data obtained nearly exclusively starting in 1978 and including no pre-1976 data. The NIOSH EO exposure model predicted a decreasing trend in sterilizer operators EO exposures (worker who were most heavily exposed to EO) that continued in trend from 1978 to 1980 toward in primary that model by decreasing sterilizer chamber gas back in time prior to 1978. This NIOSH-predicted trend was based only on a post-1978 statistical correlation without independent verification using any pre-1976 measurements or consideration of the EO sterilization equipment or operation (EOSEO) prior to 1978. A new physical model was developed using empirical data from published literature bearing on EOSEO, historical data on EO kill concentrations, measured EO residue levels in sterilized materials, data on EO concentrations in a sterilization chamber following one or multiple washes, and information on routine operator practices from highly experienced sterilizer operators from the pre-1978 period. Model estimates were benchmarked against the NIOSH estimate for 1978/80 EO concentration estimates. The ex-posure levels for jobs with the maximum concentrations were estimated to be more than 5-fold greater than NIOSH-estimated exposures between 1938 and 1960. In other words, modeled concentrations using physical/engineer- ing information contradict NIOSH estimates that workers experienced lower EO concentrations from the mid-1930s through the 1960s compared to those measured in 1978. Because underestimated exposures generally lead to over-estimated cancer risks, more detailed and empirically based assessment of historical EOSEO and resulting EO exposures experienced by sterilizer workers is required for a realistic EO cancer risk assessment.

1856 Hazard Characterization of Acrylates by In Vitro and Ex Vivo Models—An Update of the ExiTox Project

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ExiTox (Explain Inhalation Toxicology) aims to develop an integrated approach for the hazard characterization of inhalable compounds without in vivo animal testing. Different in vitro and in silico models are developed by assessing groups of compounds that share structural properties and induced a shared toxicological effect pattern in existing in vivo studies with repeated exposure via inhalation. The shared toxicological effects in these read-across groups might indicate a shared mode of action. We hypothesize that in vitro data can be used to strengthen the read-across hypothesis e.g. by showing shared adverse outcome pathways. We selected a group of four aliphatic acrylates, which induced inflammation and hyperplasia in the respiratory tract of the test animals at dose levels ranging from 15-25 ppm. These acrylates were tested submerse in A549 cells and human precision-cut lung slices (PCLS) dose-dependently. Exposure occurred over three days for one hour. In A549 cells the cytotoxicity of acrylates was assessed with EC50 values of 2.15, 3.7, 1.12 and 1.57 mM for methyl acrylate, ethyl acrylate, propyl acrylate and butyl acrylate, respectively. Comparable to cells, PCLS showed similar sensi-tivity with the EC50 values of 1.61, 3.02, 2.99 and 4.48 mM for methyl acrylate, ethyl acrylate, propyl acrylate and butyl acrylate, respectively. The bioinformatic process chain includes analysis of whole transcriptional RNA-seq data and the use of tools and technology of all compounds to detect the expression changes, focus on regul-ation via enhancers and underlying miRNA levels, involves network analysis to identify potential biomarker up to a postulated diagnostic profile for each specific read-across group. The results for DEG and miRNA will be presented. This work was supported by BMBF grant FKZ 031A269A-D.

1857 Estimating the Risk of Long-Term Health Effects following Acute Exposure to Cholinergic Agents

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The acute effects of many chemicals are well described with supporting data through traditional toxicology studies. In contrast, long-term health effects resulting from a single acute exposure are sparsely supported by epidemio-logical studies of workers exposed to toxic agents. In this study, we used the International Cholinergic Agents (ICA) project 50 year data set to develop a risk estimation approach to identify potential biomarkers up to a postulated diagnostic profile for each specific read-across group. The results for DEG and miRNA will be presented. This work was supported by BMBF grant FKZ 031A269A-D.

1858 Derivation of a No Significant Risk Level (NSRL) for Acrylamide

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Acrylamide is included on the State of California’s Proposition 65 list as a carcinogen based on evaluations by authoritative bodies (International Agency for Research on Cancer (IARC) and US Environmental Protection Agency (US EPA)). Acrylamide is mainly used as a monomer in the synthesis of polycrylamides, but is also found in cigarette smoke and hence indoor air. More recently, acrylamide was found to be generated in carbohydrate-rich foods processed by standard cooking methods such as baking, frying and roasting. As a result, acrylamide is found in many types of foods, including breads, cereals, coffee, cookies, French fries, and potato chips. In 1990, California’s Office of Environmental Health Hazard Assessment (OEHHA) established a no signifi-cant risk level (NSRL) of 0.2 µg/day for acrylamide based on a cancer potency factor established by US EPA. OEHHA proposed an updated NSRL of 1 µg/day in 2008, and withdrew it in 2009. Since then, multiple cancer studies, mode of carcinogenic action studies and reviews have been published, includ-ing, in 2010, an updated carcinogenicity assessment published by US EPA’s Integrated Risk Information System (IRIS). Additionally, in 2012 the National Toxicology Program (NTP) completed lifetime bioassays of acrylamide car-cinogenicity in rats and mice. The objective of this project is to develop an updated NSRL for dietary exposure to acrylamide. A comprehensive literature review identified 50 models are developed by...
review indicated that the mouse is the most sensitive species to the carcinogenic activity of acrylamide. Using benchmark dose modeling and combining the incidences of multiple tumors, cancer slope factors (CSFs) of 0.481 (mg/kg/day)\(^{-1}\) and 0.33 (mg/kg/day)\(^{-1}\) were derived based on increased incidences of neoplastic lesions in Harderian gland, lung, and stomach in male mice and Harderian gland, lung, mammary gland, ovary, skin, and stomach in female mice, respectively, as reported in the NTP chronic bioassay. Both CSFs were converted to the same human equivalent CSF of 0.61 (mg/kg/day)\(^{-1}\) using a pharmacokinetic (PK) scaling approach based on physiologically based pharmacokinetic (PBPK) modeling. This CSF was used to derive a NSRL of 1.1 μg/day at the cancer risk level of 1 in 100,000 (10\(^{-5}\)). When it can be demonstrated via quantitative exposure assessments, that the daily consumption of exposure to acrylamide is below the NSRL, industries are exempt from the Proposition 65 labeling requirement and drinking water discharge prohibition.

### 1859 Risk Assessment of Poly Chlorinated Biphenyls (PCBs) Levels in Water, Sediment and Edible Biota from a Contaminated Tropical Ecosystem

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Over the years, contamination of aquatic ecosystems with persistent organic pollutants (POPs) has been an issue of global concern due to the potential for ecological and human health risk. This study assessed the risk associated with the levels of PCBs in surface water, sediment and Chrysophyta nigroditatus from Agboyi Creek, South-west Nigeria using a Hewlett Packard GC 5890 Series 11. The study detected 27 PCB congeners in all examined samples in the order; water > sediment > fish. Σ-penta-PCBs accounting for 19% of total PCBs recorded was the most dominant in all examined samples. The levels of total indicator PCBs (Σ-PCBs) in sediments (0.116 to 229.38μg/kg) exceeded the Canadian Sediment Quality Standard threshold effect level of 0.03μg/kg and the National Oceanic Atmospheric Administration threshold effect level (NOAA TEL) for fresh and marine sediments indicating high ecological risk. Furthermore, the levels of the non-ortho-substituted coplanar PCBs and estimated toxic equivalent quotient (TEQ) which were higher than NOAAs fish toxic equivalence factors (TEF) in fish suggest the potential for adverse effects in populations with high consumption rate. This calls for effective policies and stringent regulation for the protection of ecosystem and human health.

### 1860 Concordance of Dose-Response Modeling with Critical Effect LOAEL in the Derivation of an Oral Reference Dose for 2,4-Pentanediene

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General population exposure to 2,4-pentanediene (2,4-PD) may occur from its use in paints, cleaning agents, solvents and coatings (some of which have drinking water contact applications). Exposure to 2,4-PD can also occur through inhalation as 2,4-PD is permitted for indoor and outdoor use. An oral risk assessment was needed to establish allowable drinking water levels. Absent any human health effect data, hazard identification relied on laboratory animal data, which identified treatment-related effects following oral or inhalation exposure including frank effects (mortality), generalized systemic toxicity (evidenced by reduced body weights), neurotoxicity, thymic toxicity, and fetotoxicity. Oral-route repeated dose toxicity data for 2,4-PD were limited to non-standard short-term studies (≤15 days). In a subchronic inhalation study in F344 rats, 2,4-PD caused significant mortality at the high exposure level of 29 mg/kg/day and BMDL\(_{03}\) of 18 mg/kg-day were determined from the available studies. In a subchronic inhalation study in F344 rats, 2,4-PD caused significant mortality at the high exposure level of 29 mg/kg/day and BMDL\(_{03}\) of 18 mg/kg-day were determined from the available studies. The critical effect identified from the available studies was fetotoxicity at 202 ppm (human equivalent dose of 29 mg/kg-day). Using benchmark dose modeling (BMD), a BMD\(_{05}\) of 29 mg/kg-day and BMD\(_{01}\) of 18 mg/kg-day were calculated for the decrease in male fetal weights (litter based). Following US EPA BMD guidelines, a benchmark response (BMR) of 3% was selected, as it is ideal to have empirical data points at or near the BMR. The fetal weight decreases of 0.6% at 7.8 mg/kg/day and 3% at 29 mg/kg/day served as the basis of the no- and lowest-observed adverse effect levels, respectively, defined based on statistical significance compared to controls. Confidence in the BMD modeling is demonstrated by the concordance of the BMD and the LOAEL for the critical effect. Using a total uncertainty factor of 1000x (3x interspecies, 10x intraspecies, 3x subchronic, 10x database), an oral RFD of 0.018 mg/kg-day was determined for 2,4-PD. The resulting Total Allowable Concentration in drinking water was 100 μg/L.

### 1861 Cefcr LRI AIRM-8: Prediction of STOT-RE Classification by New Approach Methodologies

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The AIRM-8 project aims to assess the ability of in vitro data from the Tox21 program to predict STOT-RE categories of a range of chemicals. The application of New Approach Methodologies (NAMs) in risk assessment is an area of intensive research. AIRM-8 aims to advance the understanding of the use of NAMs by analysing a different way of predicting systemic toxicity, especially the STOT-RE classification. STOT-RE classification is based on the NOAEL of the in vivo study and does not consider the type of effect or the organ affected. If prediction of STOT-RE classification by NAMs is possible, this will contribute to a paradigm shift in risk assessment and will motivate the use of NAMs in prioritisation and labelling, and eventually in safety assessment as well. STOT-RE classifications were gathered and derived from different sources e.g. from a set of 90 day studies with repeated oral exposure (extracted from the RepDose/ToxRef/Hess and Cosmos databases) and the inventory of harmonised classifications was parallel, with the AC50 values from Tox21 and considered all values occurring at sub-cytotoxic concentrations. The sparse data matrix of the 43 individual assays was aggregated to seven categories representing six toxicity pathways and cytotoxicity values. The intersection of both the in vivo and in vitro data resulted in a data set of 749 compounds. For large-scale in-vivo extrapolation (IVE) relevant data such as plasma protein binding, renal and hepatic clearance were identified from existing data sets and the literature. Prior to in silico profiling, the structural information was quality controlled and corrected. From a range of statistical methods, k-nearest neighbours (kNN) and random forest (RF) were selected as the models for prediction of the classification.

In addition, seventeen read-across groups were defined. The grouped compounds share structural characteristics and specific/unspecific in vivo apical findings/target organs. Groups were distinguished for which a shared toxicological effect pattern might be indicative of a shared mode of action from those with unspecific toxicological effects e.g. weight changes or no toxicological effects. In these read-across groups, we analysed the mechanistic links between the in vitro results and the in vivo apical endpoint leading to STOT-RE classification. Financial support for this work was provided by the CEFIC Long Range Research Initiative (CEFIC LRI AIRM-8).

### 1862 Toxicity of JUUL Fluids and Aerosols Correlates Strongly with Nicotine Concentrations

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JUUL EC products have become very accessible and popular among middle school and high school students. The purposes of this study were to: (1) identify and quantify flavor chemicals in JUUL EC pod juice flavors purchased online and in stores and (2) determine if these products are cytotoxic using in vitro models. Eight JUUL pod flavors were analyzed using GCMS to identify and quantify flavor chemicals in pod juice and aerosols made at variable flow rates. BEAS-2B cells were exposed to fluids and aerosols for 24 hrs and cytotoxicity was determined using methyl tetrazolium (MTT), and neutral red assays. 58 flavor chemicals were identified in all the products with three flavors (menthol, ethyl maltol, and vanillin) being detected at concentrations greater than 1mg/mL. Duplicate pods had very similar flavor chemicals compositions. Transfer efficiency of total flavor chemicals in the pod juice into aerosols was generally greater than 75%. Nicotine concentrations generally exceeded 50mg/mL. Both cytotoxicity assays generally revealed very similar patterns of response with the most cytotoxicity revealed at 3% and 10% for cells treated with fluids. At lower concentrations, toxicity was observed with only the MTT assay for 3 of 8 flavors. Very slight levels of LDH leakage into extracellular medium was observed after treatment with aerosols. The toxicity of the aerosols in MTT and neutral red assays was observed between 0.4% - 3% when compared to the fluids. Fluids and aerosols from JUUL products are cytotoxic and affect cell survival. These data provide new and useful information for users and regulatory agencies on the chemical composition of JUUL products and their potential to cause harm to users.
This review compares exposure and alarm limits for carbon monoxide (CO) in the US and Europe and proposes a new basis for regulating CO. The customary ranking of CO limits by the maximum (max) concentration allowed is contrasted with ranking by the total exposure allowed, calculated as the product of the max allowed concentration and max allowed exposure time in ppm*hours (p*h). This analysis shows that multi-tier CO standards allow 2 to 5 times more exposure from lower concentrations of CO over longer times than the US maximum exposure to higher levels. No support for this bias is found in the literature. The WHO, for example, allows total CO exposure up to 146 p*h when the concentration is 7 mg/m³ (6.1 ppm) but only up to 22 p*h at 100 mg/m³ (87.3 ppm). The UL 2034 standard for home CO alarms, in contrast, allows up to 100 p*h at the highest alarm level (400 ppm) but 280 p*h at the lowest (70 ppm). The US NIOSH recommends the same 280 p*h for the maximum exposure of workers on 8-hour shifts, while the US OSHA permits up to 400 p*h per shift—the highest of any CO standard. CO alarm standards also specify a concentration below which even continuous exposure is considered harmless and should never trigger alarms: 70 ppm in US and 50 ppm in Europe. But these thresholds are not supported by the literature, which includes over 100 environmental epidemiology studies reporting statistically significant increases in adverse outcomes associated with increases in average hourly or daily CO of 1 ppm or less against background CO levels typically around 5 ppm. Most CO alarm and exposure limits are still based on the results of controlled exposure studies. In some cases, the historical basis for these limits was weakened by the realization that regulators mistakenly believed the adverse effects of CO were mediated by blood carboxyhemoglobin (COHb) and could be prevented by keeping levels under 2, 5 or 10%. It is now clear that CO poisoning outcomes do not correlate with COHb, so some new basis for setting exposure and alarm limits is needed. According to the epidemiology literature, fetuses and infants are most vulnerable to CO: they face higher risks from average increases in CO of 1 ppm or less over 1 hour to 1 day that are 1 to 2 orders of magnitude greater than the risks faced by adults. Protecting them will require lowering CO exposure and alarm limits over 10-fold. For the health of everyone, home CO alarm standards also should account for the time delays that currently allow high exposures to occur repeatedly without warning. Only immediate CO alarms can actually prevent CO poisoning.
An In Vitro Human Assay for Evaluating Immunogenic and Sensitization Potential of Personal Care and Cosmetic Products


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The recent ban and calls for reduced testing of cosmetics on animals present a unique challenge for product safety evaluation. With the reduction or elimination of animal testing, manufacturers are left with limited options, as fewer robust in vitro tests are available and human studies are costly. The purpose of this study was to evaluate the safety of a hair cleansing conditioner by utilizing a novel in vitro human skin test with high specificity and sensitivity for assessing immunogenic and sensitization potential. Peripheral blood mononuclear cells (PBMCs) and human skin biopsies were obtained from healthy volunteers and monocyte derived dendritic cells (MoDCs) were generated. MoDCs were primed by hair cleansing conditioner for 24 hours and then co-cultured with autologous lymphocytes and dendritic cells for 4 days. After the 4 day culture, primed MoDCs were cultured with skin biopsies for 3 days. The skin biopsies were then fixed, sectioned, and stained for histopathological evaluation. Histological damage was graded from 1 to 4; grade 1 represents a negative result for tissue damage and grade 2 or more represents a positive result for tissue damage. Additionally, T cell proliferation and IFNγ levels were determined. The results of this study showed that treatment of PBMCs with the hair cleansing conditioner resulted in acceptable cell viabilities. Treatment of MoDCs with the hair cleansing conditioner or negative controls did not lead to increased T cell proliferation, whereas the positive control treatment did significantly increase T cell proliferation. Human skin biopsies exposed to the negative control and cleansing condition remained undamaged (grade 1), whereas exposure to the positive control resulted in tissue damage (grade III). Together, these results demonstrate that the hair cleansing conditioner did not elicit an immunological or sensitization response at the concentration tested. The results of this study demonstrate that this in vitro human assay is applicable and well suited for personal care and cosmetic product safety evaluations.

Fit-for-Purpose In Vitro Assays in Risk-Based Decision Making—Example with Estrogenic Compounds


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Chemical risk assessment relies on expensive in vivo studies, and the current scale of animal testing is insufficient to address chemical safety concerns as regulatory agencies require more complete toxicity data. We demonstrate a practical tiered testing approach to reduce animal use by employing multiple levels of risk prioritization. A fit-for-purpose uterine estrogen assay was coupled with in silico and high-throughput in vitro to in vivo extrapolation (HT-IVIVE) to improve prioritization of estrogenic compounds and support in vitro to in vivo hazard characterization in a tissue-specific context. Initial prioritization was based on activity in estrogen receptor (ER) dependent Toxtast assays. To assess uterine-specific reactivity, 112 Toxtast-prioritized compounds were screened in the uterine estrogen response assay for ER-mediated proliferation in Ishikawa cells, a human uterine cell line. Estimates of activity (AC50s) were based on dose-dependent proliferation, and quantitative in vitro to in vivo extrapolation (Q-IVIVE) was used to predict a human equivalent dose (HED) for each compound. Estrogen response assay-derived HEDs were compared with in vivo points of departure from existing uterotrophic assay and two-generation reproductive toxicity studies. Pearson correlation (in log-space) between in vitro HEDs and in vivo points of departure was 0.47. Estrogen response assay-derived HEDs were lower than in vivo points of departure in 37 (93%) of 40 compounds active in both models. Of 56 compounds active in in vivo studies, 16 (29%) were inactive in the estrogen response assay, possibly due to differences in metabolism in the two test systems. Our positive control, the synthetic estrogen 17alpha-ethinylestradiol, produced mean HEDs of 0.56, 0.030, and 0.0030 µg/kg-day in the Toxtast assays, estrogen response assay, and in vivo studies, respectively. These results support the use of fit-for-purpose assays to refine chemical risk prioritization based on the estrogen response assay’s range of detection and characterization of a potent ER agonist, which are improved over Toxtast assays. Developing well-accepted and more efficient in vitro assays and IVIVE methods is crucial for the proper interpretation of in vitro hazard data for health risk assessment.

Human Health Risk Assessment of Heavy Metals in Children’s Foods

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Concerns have recently been raised about the presence of heavy metals in children’s foods following analyses by Consumer Reports, a consumer watchdog, and the Environmental Defense Fund (EDF), a nonprofit environmental advocacy group. Although Consumer Reports has not made its dataset publicly available, the EDF presented its analysis based on the US Food & Drug Administration’s US FDA evaluation of foods commonly eaten by infants and toddlers, which reported levels of lead (Pb) and cadmium (Cd) in approximately 400 samples of children’s foods. In their analysis, they did not perform a risk assessment, EDF suggests that heavy metals in foods are health risks. The objective of our study was to determine whether the detected levels of Pb and Cd in the US FDA’s evaluation posed any health risks to children. Using the average food intake rates of children described in the US Environmental Protection Agency’s (US EPA) Exposure Factors Handbook (EFH), the US EPA reference dose (RfD) for Cd, and the California Office of Environmental Health Hazard Assessment’s (OEHHA) maximum allowable dose level (MADL) for Pb, we calculated hazard indices (HI) for daily consumption of each metal in children’s foods. We additionally calculated lifetime cancer risk estimates for the consumption of metals in children’s foods based on the OEHHA’s oral slope factor for Pb. The HI were less than 1 across the ten food product categories we considered, with the highest HI in juice boxes and pouches (0.46) and peanut butter (0.14) for Cd. The cumulative lifetime risk estimates for each of the product categories were less than 1.0x10^-6 (3.2 x10^-5 to 1.5 x10^-6), indicating that there would not be an increased lifetime cancer risk from exposure to Pb in foods consumed during childhood. Overall, the results of our analysis suggest that a child’s typical intake of these foods would not lead to adverse health effects from Pb or Cd.

Considerations for Grouping Different PFAS Together to Develop Guidance Values


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Regulatory agencies worldwide have taken an interest in developing acceptable levels of low- and polyfluoroalkyl substances (PFAS) in milk drinking water. Because there are so many different PFAS, it is challenging to generate values that are based on toxicokinetic and toxicological studies of each individual chemical. Some agencies have approached this challenge by grouping PFAS together and setting one limit for the combined concentrations of the chemicals. For example, US EPA set a combined drinking water health advisory of 70 parts per trillion (ppt) for the sum of perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS). For PFOA, PFOS, perfluorohexane sulfonate (PFHxS), perfluorononanoic acid (PFNA), and perfluorohexanoic acid (PFHxA) combined, the Massachusetts Department of Environmental Protection set a drinking water value of 70 ppt, while the Vermont Agency of Natural Resources set a combined value of 20 ppt. We conducted an analysis of the toxicological and kinetic data for six perfluorinated chemicals, including perfluorobutanoic acid (PFBA), perfluorobutane sulfonic acid (PFBS), PFHxS, PFNA, PFOA, and PFOS, with the aim of determining whether grouping of any of these PFAS for regulatory purposes is scientifically sound according to standard US EPA methodology. Both the critical toxicological effects identified by regulatory agencies and the kinetics differ among the PFAS, which limits the utility of grouping them together for regulatory purposes. For PFOA, PFOS, and PFNA, the endpoints are developmental. Agency-identified critical effects for PFHxS are body weight and serum lipid levels, and increase in prothrombin time. Decreased cholesterol has been identified as the critical effect for PFBA, and kidney histopathology and blood changes for PFBS. Regarding toxicokinetics, shorter-chain PFAS such as PFBA and PFBS have substantially shorter half-lives in the human body than the longer-chain PFAS. Based on our analysis, we concluded that PFAS should only be grouped together for the setting of regulatory guidelines if the compounds have both similar endpoints for toxicity and similar half-lives. According to US EPA methodology, PFOA, PFOS, and PFNA are appropriately grouped together based on similar critical effects and half-lives but PFBA, PFBS, and PFHxS should not
be grouped with PFOA, PFOS, and PFNA or with each other. Our analysis pro-
vides guidance for setting science-based regulatory levels for PFAS in drink-
ing water.

1871 Risk Assessment to Determine Proposition 65 Compliance for a Consumer Product: Diisononyl Phthalate
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This risk assessment estimated the potential consumer exposure to diisononyl phthalate (DINP, CAS 28553-12-0) associated with a foam material present inside an upholstery cushion. The focus of the evaluation was California’s Proposition 65, which requires product warning labels if products contain certain hazardous chemicals above regulatory limits. DINP has been listed as a carcinogen to the State of California to cause cancer under Proposition 65. The No Significant Risk Level (NSRL) of 146 micrograms per day is based on liver cancer and leukemias observed in rodent studies. Product chemical analysis indicated the foam inside the upholstery cushion exceeded the Consumer Product Safety Commission limit of 0.1% (1,000 parts per million) for phthalates; however, it was unclear whether exposure to this chemical would exceed the Proposition 65 NSRL under typical product use conditions. Our evaluation of potential consumer exposure to DINP consisted of two steps: estimating each route-specific exposure concentration on a micrograms per day basis and then comparing the dose estimates to the NSRL. The routes of exposure evaluated were dermal, inhalation, and oral, although the last was considered highly unlikely. We relied on US EPA and California EPA sources for general exposure parameters and on previously published studies that measured migration of DINP from upholstery materials to adjacent environmental media. The assessment was conservative, erring on overestimating exposure when uncertainties were present for particular assumptions used in the calculations. When we compared our exposure estimates to the California Proposition 65 NSRL, the results indicated that exposures to DINP in the foam were below the NSRL, specifically 1.6 to 3,200 times lower depending on the particular exposure scenario considered. These results suggested that the product was in compliance with the Proposition 65 Safe Harbor provisions.

1872 Evaluation of the Carcinogenic Mode-of-Action for Tetrachloroethylene and Propylene for an Occupational Exposure Limit
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Tetrachloroethylene (PCE) is classified by the United States Environmental Agency (US EPA) as “likely to be carcinogenic in humans,” based primarily on evidence of increased liver tumors in mice. Evidence of an association between PCE exposure and cancer in humans is inadequate or limited at best, with few studies showing positive associations, and for those studies that do, exposure estimates are lacking and/or confounded by exposure to other potential carcinogens. The metabolism of PCE yields multiple metabolites through two main pathways: (1) the “oxidative pathway” via cytochrome P450 metabolism, and (2) the “glutathione (GSH) conjugation pathway” via GSH transferase activity. PCE and its main oxidative metabolite (trichloroacetic acid (TCA)) exhibit little, if any, genotoxicity. The GSH metabolizers (trichloro-

1873 Rapid Evidence Mapping for Health: A Case Study
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Stakeholders in the field of environmental health are increasingly relying on tools and practices from the disciplines of evidence synthesis and systematic review to summarize the literature and identify scientific consensus with respect to potential health risks. Given the ever-accelerating pace of publications in this field, the practice of “evidence mapping” is being increasingly used to identify the key areas of study relevant to a given topic along with gaps in the literature. However, constructing detailed evidence maps can be resource-intensive, limiting their utility for practical implementation, particularly for broader questions of interest. As a result, approaches that increase the speed and reproducibility of evidence mapping are in great demand. Here we outline a process called “rapid evidence mapping” (REM), which we define as a resource-efficient form of knowledge synthesis in which components of the systematic review process are simplified to produce a rapid and qualitative representation of the scientific evidence. We show how rapid evidence maps can be created with the aid of Sciome’s text-mining and machine learning software, and to illustrate the application of the procedure, we describe a proof-of-concept case study on the topic of low-calorie sweeteners (LCS) with respect to human dietary exposures and health outcomes. The resulting REM produced similar findings compared to a traditional evidence mapping of the same topic (Wang et al., 2016), but required significantly less time, effort, and resources to create. Furthermore, a sensitivity analysis evaluating the set of studies included at 25% recall (i.e., the point at which the machine learning algorithms predicted we had identified 25% of all relevant references) would have resulted in the same conclusions regarding the current state of the science and existing research gaps. This observation suggests that further efficiency gains can be achieved by mapping only a computer-selected subset of the available literature. REMs can be used to quickly summarize the available body of evidence relevant to a research question, identify gaps in the literature to inform future research, and contextualize the design of a systematic review within the broader scientific literature, significantly reducing human effort while yielding results comparable to those from traditional methods. The potential time savings of this approach make it a powerful tool for rapidly translating knowledge to inform science-based decision-making.

1874 The Norwegian Biomonitoring Study in Euromix: Comparison of Modeled Internal Exposures to BPA, BPS and BPF from Foods, Cosmetics and Thermal Paper with Urinary Concentrations
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The Horizon 2020 EuroMix project develops validated test strategies for hazard and exposure assessments of chemical mixtures. In order to examine the plausibility of source-to-dose calculations for environmental chemicals, a human biomonitoring (BM) study was performed for two periods separated by 2-3 weeks, adult volunteers (44 males and 100 females) kept detailed diaries on food consumption, the use of personal care products (PCPs) and the handling of thermal paper (TP) receipts. In parallel, 24 h urine samples were collected. Urinary levels of the bisphenols (BPs) BPA, BPS, BPB and BPF were measured for the first 24 h study period using ultra-high performance liquid chromatography and tandem mass spectrometry. BPA and BPAF were not detected. Exposures to BPA, BPS and BPF from foods, PCPs, and TP were modeled for the study population. The risk assessment tool MCRA was used for calculating individual external exposure from foods, aggregating dietary with non-dietary exposure from PCPs and TP, converting external to internal exposure using physiologically based pharmacokinetic models, and for the comparison with BM data. The selection of concentration data for exposure calculations followed two scenarios (Sc): For Sc1 samples from Norway or adjacent countries were prioritized, for Sc2 samples that had been analyzed for multiple BP analogs. In Sc1, canned fish (hake) and pasta contributed most to BPA exposure from food, while in Sc2, BPA in semi-skimmed milk and BPF in mustard were most important dietary contributors. If BP co-exposure occurred in Sc2, BPA was the main contributor for low exposures, while the BPF contribution increased with higher BP exposure. For PCPs, the median values of all BP exposure estimates in Sc1 and Sc2 were higher for women than for men, mostly with a large overlap between their 95% confidence intervals. Moisturizer, mouthwash, hairstyling products, hand soap, and hand cream were important determinants for both genders. TP had been handled by only 24 % of the participants on the first study day, and most often only once that day. Assuming single exposure to BPA and BPF from TP in Sc1 and
Acute Dermal Toxicity Studies of Aircraft Engine Oils


There is little data available on toxicity levels of used aircraft engine oils relative to their unused (new) versions. This study was conducted to determine if new engine oils and their used versions have the potential to induce dermal irritation. Twelve Male New Zealand White Rabbits (Oryctolagus cuniculus, 19 weeks old) were used to determine the acute dermal toxicity potential of Grade 3, Grade 4, GB, BDP, and 1-OHP aircraft engine oils in their unused and used states. Five fur-free test sites (6 cm² each) located lateral to the midline of the back were treated with two undiluted (0.5 ml) new engine oils and their used versions. The fifth site received RO water as a control. Each treatment was repeated 3 times (3 rabbits/oil type). Each oil was tested under both semi-occluded and occluded conditions. E-Collars were placed on each animal for at least 72 h to prevent ingestion of the test substance and/or gauze plus wrappings and disturbance of site recovery. The 4 hour exposure was followed by gauze plus wrappings removal, and gentle cleaning of sites prior to scoring for erythema and edema at 0.5, 1, 24, 48 and 72 h post exposure based on Draize (1959). Additional observations were made on days 7, 10, and 14 to determine recovery. Exposure to both used and new oils produced dermal irritation consisting of no more than slightly to well-defined erythema and very slight edema. The calculated Primary Dermal Irritation Index (PDII) indicated that all the oils were slightly irritating. Although the PDII values for new oils and their unused versions were not significantly different, they were all statistically higher (p<0.05) than those obtained for the control regardless of the type of occlusion binding applied. The used oils under semi-occlusion conditions yielded larger size effects (Cohen’s d) relative to their unused versions suggesting an enhancement in irritation when the oil is aging. Grade 4 of the used state yielded the largest size effect which was d = 5.9 versus 2.6 for its unused version. The slight dermal irritation resulting from four hours of exposure to oils raises concerns about the magnitude of impact related to prolonged and/or repeated exposure (in compliance with DODI 3216.01). Distribution A: Approved for public release; distribution unlimited (PA Case BB/AW-2018-4411; MSC/PA-2018-0279, 05 Sept 2018).

Characterizing Occurrence and Sensitization Potential of Disperse Dyes in Indoor Environments


Azobenzene-based disperse dyes used to color synthetic fabrics have been characterized as mutagens and contact allergens despite their use in clothing and detection in the aquatic environment. However, little is known about occurrences and health implications of these dyes in indoor environments. Here, we report on identifications, concentrations, and sensitization potentials of several azobenzene dyes in house dust samples collected from 190 homes in the Toddlers Exposure to SVOCs in Indoor Environments (TESIE) study in central North Carolina (2014–2016). House dust samples were collected for each household using standardized protocols. We utilized a data-dependent, suspect-screening analytical strategy to tentatively identify azobenzene-class disperse dyes in house dust. House dust sample extracts were analyzed via HPLC-ESI-HRMS/MS and searched against all compounds in CAS containing p-aminooazobenzene. Using the non-targeted analysis software Compound Discoverer, we tentatively identified >50 prominent features as azobenzene disperse dyes. Using authentic standards, we then quantified five representative azobenzene dyes (Disperse Blue 373, Disperse Orange 25, Disperse Orange 37, Disperse Orange 61, and Disperse Violet 93) and two transformation products (2,6-dibromo-4-nitroaniline and 2-bromo-4,6-dinitroaniline) in the dust samples. Dyes were quantified via HPLC-APCI-MS/MS. One or more dyes were detected in 92% of house dust samples at levels up to 3.25 pg/g house dust. Geometric means ranged from 60 ng/g to 141 ng/g house dust, with Disperse Orange 61 having the highest overall concentration. Because the intrinsic characteristic of covalent binding to proteins is a strong indicator for determining chemical sensitization potential, we used a spectrophotometric in vitro Direct Peptide Reactivity Assay (DPPRA) to measure covalent protein binding via percent peptide depletion induced by representativeazo dyes. Preliminary results demonstrate that dyes such as DISO bind to nucleophilic protein residues and induce up to 41% peptide depletion in a dose-response relationship, indicating their sensitization potential. Results suggest that exposures to disperse dyes from house dust are common and may present sensitization risk, warranting further detailed characterization.

Development of Promising Biomarkers for Prevention against Colorectal Cancer

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The incidence of colorectal cancer (CRC) has been steadily increasing, and CRC is expected to be the third most prevalent cancer in 2018 in Korea. However, the etiology of CRC is still obscure, although the consumption of red or processed meat have been declared as one of the causes. Therefore, biological monitoring can be useful to find environmental risks of CRC. We performed a pilot (N=30) and a full case-control study (N= 220; N=110 for each group) and analyzed exposure biomarkers, i.e., metabolites of polycyclic aromatic hydrocarbons and heterocyclic amines in urine, e.g., 1-OHP, PhIP, and MeIQx, and their DNA-adducts in blood, e.g., dG-C8-PhIP, and dG-C8-MeIQx, and -PhIP, by HPLC/FD or LC/MS/MS. For response biomarkers, we quantified homocysteine, malondialdehyde (MDA), and C-reactive protein (CRP) by a clinical analyzer. We also investigated their food contents by FFQ. Finally, we compared these biomarkers to the expression of CRC-related genes, e.g., PTGS2, APC, KRAS, etc., by qPCR arrays. As results, the pilot study showed higher levels of PhIP and dG-C8-MeIQx in the cases than controls (11.96±12.04 vs. 7.84±8.10 ng/mL, p=0.28; dG-C8-PhIP, 5.29±0.11 vs. 5.18±0.08 ng/L, p<0.01). Based on the pilot study, we calculated the N of subjects with power 0.8 as N=218 for the full study. We confirmed some results of the pilot study in the full study, e.g., positive associations between intakes of processed and red meat and between 1-OHP and MDA levels (p<0.01). In the full study, we found positive correlation between lipid intake and levels of MeIQx (p=0.07) and higher levels of lipid intake (g), CRP, and LDL-C in cases than controls (p<0.01). Lipid (g) intake was positively associated with the levels of MeIQx (p=0.07). However, some biomarkers, such as 1-OHP or BMI, were higher in controls than cases. To overcome the drawbacks of case-control studies, we need promising biomarkers, i.e. longitudinal, sensitive and specific biomarkers for CRC. Therefore, dG-C8-PhIP is a potential biomarker for this purpose. This study was supported by a grant (17162MFDS040) from Ministry of Food and Drug Safety in 2017–2018.

Electronic Cigarette Exhaled Aerosol Residue in Field Sites

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Electronic Cigarette (EC) users exhale aerosol which can settle on indoor surfaces forming EC exhaled aerosol residue (ECEAR). Little is known about the composition or build-up of this residue in field sites. Our objective was to identify, quantify, and compare ECEAR chemicals in two field sites: an EC user’s living room and a multi-user EC vape shop. We examined the buildup of ECEAR in commonly used materials (cotton towel, paper towel, and terrycloth towel) placed inside and outside each of the field sites. Materials were subjected to different exposure times. Nicotine, other alkaloids, and tobacco-specific nitrosamines (TSNAs) were identified and quantified in controls and field site samples using analytical chemical techniques. Nicotine and nicotine alkaloids were detected in the EC user’s living room. Concentrations of ECEAR chemicals remained relatively constant over the first 5 months, suggesting some removal of the chemicals by air flow in the living room. ECEAR chemicals were detected in the vape shop after 6 hours of exposure and levels continually increased over a month. By 1 month, the nicotine in the vape shop was 60 times higher than in the EC user’s living room. ECEAR chemical concentrations varied in different locations inside the vape shop. Control fabrics had either no detectable or very low concentrations of chemicals. In both field sites, chemicals from exhaled EC aerosols were deposited on indoor surfaces and either remained steady or accumulated over time forming ECEAR. Non-smokers, EC users, and employees of vape shops should be aware of this potential environmental hazard.
1879 Estimating the Evaporation Rate and Time-Varying Generation Rate of Acetic Acid from an All-Purpose Floor Cleaner

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Understanding the relationship between consumer product use and risk of adverse health outcomes is necessary for ensuring appropriate risk management and product stewardship. A preferred method for estimating the systemic and respiratory tract exposure and dose tailored to cleaning products use has been proposed, refining previously issued exposure guidance. The refinement suggests a tiered assessment framework that provides a margin of safety (MOS) estimate for cleaning products use and asthma responses. Tier I requires robust dose-response and exposure data. Tier II uses vetted model estimates to generate human inhalation exposure estimates, based on model inputs sourced from the publicly available and peer-reviewed literature. From these models, a reasonable worst case estimate of exposure can be generated. When high quality model inputs are not available, initial screening level values can be used, recognizing they may result in exposure estimates that greatly overestimate real-world exposures (Tier III). In situations where these overestimates exceed the MOS, refinement of the model inputs may be necessary so that a more realistic exposure estimate can be developed. We conducted in two part study to estimate and evaluate an evaporation and time-varying generation rate for acetic acid emanating from an all-purpose consumer cleaning product formulation used to mop floors. An evaporation rate and time-varying generation were estimated from chamber studies using a small spill model. The Well Mixed Room (WMR) and Near Field Fair Field (NF FF) models, informed by these generation and and generation rates were used to predict airborne acetic acid concentrations evaporating from hardwood floors in an all-purpose room and tile floors in a bathroom. Model predicted concentrations were compared with the measured concentrations collected during a field study. WMR and NF modeled concentrations were comparable, suggesting evaporation and generation rate portability. Further, these rates will support Tier II exposure assessments.

1880 Reassessing Drinking Water Exposures: Relevance of Reactive Electrophiles Formed in Drinking Water Disinfection

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Worldwide, drinking water treatment depends heavily on the use of disinfectants. Unfortunately, the reaction of disinfectants with organic and inorganic water constituents can also result in the formation of (potentially) carcinogenic disinfection by-products (DBPs). Phenolic compounds are of particular relevance as DBP precursors as they are commonly found in many anthropogenic chemicals, including pesticides, flame retardants, plasticizers, pharmaceuticales and personal care products, as well as natural organic matter. While the fate of phenols during reaction with chlorine has been investigated for decades, there is still considerable uncertainty regarding the identity of DBPs that are formed at elevated chlorine exposures, i.e., under conditions typically encountered during disinfection of drinking water. This also holds true for newer treatment technologies such as the application of UV light with and without the addition of hydrogen peroxide with the latter utilizing OH-radicals as strong oxidants. To address this knowledge gap, we used a novel approach that focuses on the identification of reactive electrophiles by investigating the formation of adducts with nucleophilic biomolecules. Similar approaches have previously been used for the identification of toxic metabolites during xenobiotic metabolism. Using the amino acid N-acetyl-lysine and N-acetyl-cysteine as nucleophilic targets, we were able to identify the formation of previously unknown electrophilic dicarbonyl compounds during the reaction of phenol with chlorine and OH-radicals. Experiments with a variety of alkyl-, chlorine- and bromine-substituted phenols revealed that the formation of these ring cleavage products strongly depends on the position of the substituents relative to the hydroxyl group with yields varying between 0 - 45%. The high genotoxicity and mutagenicity of the formed dicarbonyls compounds indicate that they can contribute substantially to the overall toxicity of disinfectated waters if phenolic compounds are present. The results further highlight the suitability of the used approach to specifically identify the formation of electrophilic transformation products that are formed during water disinfection.

1881 The Missing Link: Using the TTC to Provide a Risk-Based Approach for Focusing Non-targeted Analysis Efforts

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Resources are increasingly being allocated to apply advanced analytical chemistry methods in a discovery science mode for non-targeted analysis (NTA). NTA and suspect screening analysis (SSA) methods can detect and potentially identify chemicals in various media (e.g., household dust, water, blood, etc.). Yet detection, using advanced analysis with exquisitely low detection limits, is not a surprise. The overarching question, “what does the presence of a chemical in a SSA/NTA study mean in terms of potential effects on health?” cannot be answered from an SSA/NTA study alone because the methods to link SSA/NTA information to exposure to potential risk have not yet been systematically developed. Because SSA/NTA is discovery science, efforts to identify exact structures can be challenging and require considerable resources. Even when a structure is identified, toxicity and exposure data are often lacking. To address these challenges, we propose a tiered method for integrating emerging SSA/NTA data within exposure and risk-based prioritization frameworks. As a first tier, the Threshold of Toxicological Concern (TTC) can be used in conjunction with simple exposure scenarios to interpret and guide SSA/NTA information. For example, the TTC Cramer Class III intake rate of 1.5 μg/kg-day can be combined with US EPA exposure factor handbook drinking water intake rates (e.g., 2 L/day; 80 kg bw) to determine the concentrations in drinking water that correspond to the TTC. In this case, drinking water concentrations less than 60 μg/L in water would not be expected to produce any level of effect (e.g., threshold of effect or exposure) by combining the TTC of 1.5 μg/kg-day with US EPA median estimates for household dust ingestion rates (e.g., 30 mg/day; 80 kg bw), a chemical concentration less than 4 g/kg in dust would not be expected to pose a systemic toxicity risk over a lifetime of exposure. As a second tier this TTC-based approach can be extended to other exposure media (e.g., indoor air) using multi-media mass balance exposure models for further integrating exposures via multiple pathways and for a suite of different sub-populations. Scenarios can be tailored to specific populations, exposure routes and environmental media to focus SSA/NTA activities and resources on identifying and quantifying substances exceeding such risk-based exposure values. The TTC-based framework provides, as a first approximation, a means to interpret and prioritize SSA/NTA studies and emerging data in a risk-based context.

1882 Testing Strategy Development Based on Effects of Low-Level Mixtures of Chemicals in Drinking Water (Sources) in Reporter Gene Assays

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A plethora of in vitro assays is developed for chemical risk assessment and clinical diagnostics to test effects of individual chemicals and mixtures on different biological processes. In this research, effect-based in vitro reporter gene assays are used to detect effects of realistic low-level mixtures of known and emerging chemicals in water. CALUX reporter cell lines with the luciferase gene coupled to responsive elements detect ligand-specific receptor binding. This is measured as light emitted (luciferase expression results in light emissions) in addition of substrate and benchmarked against a reference chemical response. Possible cytotoxic effects are evaluated using the cytotox CALUX bioassay which constitutively expresses luciferase. A relevant battery of CALUX assays detecting effects of environmental mixtures of chemicals present in water sources is selected in a tiered approach. Effects of low-level mixtures of chemicals extracted from water sources from 8 European countries (Germany, France, the Netherlands, UK, Greece, Hungary, Poland, Switzerland), Israel and India were tested in 17 receptor-based CALUX assays. Some specific in vitro effects (anti-estrogenity, progesterone receptor activation and p53 activation) were only rarely induced, while dioxin receptor-activation was observed almost independently of water type. Other effects were observed variably [PXR activation, estrogenicity, (anti-)androgenicity, glucocorticoid receptor activation, PPAR receptor activation and Nrf2 pathway activation] and were in most cases reduced following water treatment. CALUX analysis results indicated that considerable (regional) differences in drinking water quality from various water sources exist. A battery of effect-based in vitro bioassays as integrated risk measure (detecting the net effect of mixtures of chemicals with the same mechanism-of-action) can be used to benchmark differences and variation in human exposure to low-level mixtures.
Exposure of Third Trimester Human Fetuses to Persistent Environmental Chemicals

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Exposure to persistent environmental chemicals such as organochlorine pesticides (OPs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and perfluorinated alkyl substances (PFASs) during gestation has been associated to adverse effects on fetal growth and development. Typically, fetal exposure is estimated via analysis of chemicals in maternal serum samples. Whether maternal serum concentrations give a good estimate of fetal tissue exposure levels is not known. We have analyzed the levels of 9 OPs, 10 PCBs, 3 PBDEs and 6 PFASs in autopsy samples collected from 38 stillborn infants (gestational age 30-42 weeks) undergoing cause of death investigation. The samples included maternal serum, placenta, fetal liver, lung, heart, adipose tissue and brain. The study was approved by the Regional Ethical Review Board in Stockholm. Nine of the 28 evaluated chemicals were found over the limit of quantification in every maternal serum sample and 22 in every fetus. The highest chemical load, 58 ng/g tissue (56% p,p'-DDE; 7% PCB-153, 6% PCB-138), was found in adipose tissue followed by 8 ng/g (19% p,p'-DDE; 17% beta-HCH, 17% PFOS) in liver. To study fetal exposure in relation to maternal serum levels, tissue:serum ratios were calculated. The ratios varied from 0.04 to 4.55 and were significantly lower for PFASs compared to the rest of the chemicals. Further, some of the ratios were modified by fetal sex, being higher in males compared to females. In conclusion, all fetuses were intrinsically exposed to mixtures of persistent environmental chemicals with the highest burden being present in adipose tissue. Transfer of the chemicals from maternal serum to fetal tissues varied significantly and was modified by fetal sex. Altogether, our results suggest that maternal serum levels of chemicals give a poor estimate of actual fetal tissue exposure levels.

Association between Maternal Exposure to PCBs and Head Circumference of Male Newborns at Birth in Chiba Birth Cohort (C-MACH)

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In a previous study, we reported that maternal exposure to polychlorinated biphenyls (PCBs) was negatively correlated with head circumference at the birth of the newborns. The objective of this study was to see if there were sex differences in the relationship between fetal exposure to PCBs and the body size of the newborns at birth. We used the data of 93 mothers and their singleton infants (born at 36-41 week's gestational age) participated in a birth cohort study, Chiba Study of Mother and Child Health (C-MACH), focusing on environmental health effects on fetuses. After excluding missing data, the data of maternal blood serum from 71 pairs (male newborns (n=29) and female newborns (n=42)) and the data of cord serum from 68 pairs (male newborns (n=28) and female newborns (n=40)) were analyzed. Concentration levels of 13 PCBs, 11 PFASs serum around at 32 weeks gestational age and cord serum were analyzed. Body size of the newborns, which included body weight, length, head and chest circumferences were collected from medical record at delivery. Multiple regression analysis was performed to examine the relationship between the levels of total PCBs and body size in male and female newborns respectively. The level of total PCBs in maternal serum was negatively correlated with head circumference significantly in male newborns (B: -1.18, 95%CI: -2.03, -0.34, p<0.01) but not in female newborns (B: -0.74, 95%CI: -2.58, 1.10, p=0.419) in the model adjusted for potential confounding factors. Also, the level of total PCBs in cord serum was not associated with head circumference significantly. As a result, the sex differences were not found between maternal exposure to PCBs and body size except for head circumference. These findings suggest that maternal exposure to PCBs might affect the head circumference in male newborns. However, the sample size in this study was relatively small, so further study in larger population would be necessary.
1887 Estimated Intakes of Hydroxyanthracene Derivatives (HADs) from Consumption of Drinkable Aloe Vera Products on the EU Market and Foods Consumed in the Background Diet

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The European Food Safety Authority (EFSA) recently raised safety concerns over evidence of genotoxicity and carcinogenicity of HADs in food supplements used as laxatives on the EU market but was unable to establish a safe intake level. HADs naturally occur in vegetables, spices, herbs and other botanicals, including Aloe vera, which is used in foods and used for medicinal purposes. Due to a lack of available data, EFSA could not estimate unintentional HAD exposure from these sources in the normal diet. The primary HADs found in Aloe vera leaves are aloins A and B. The purpose of this study was to estimate the intake of aloins A and B from drinkable Aloe vera products sold on the EU market which are not intended for laxative use, as well as the intake of known HADs from the normal diet, to determine whether exposure to HADs from these products is of concern. The levels of aloins A and B were analyzed in 15 drinkable Aloe vera products (gels, juices or drinks) from 6 European countries. Maximum potential exposure from each product was calculated based on the daily recommended serving on product labels. Dietary sources of HADs identified in the literature. Exposure to HADs from the normal diet was assessed using the reported concentrations of HADs in food in combination with food consumption data from the EFSA Comprehensive Database (2015) and 3 individual European consumption surveys (from the UK, France and the Netherlands). The estimated intakes of aloins A and B from drinkable Aloe vera products ranged from <1 to 864 µg/person/day. Exposure to HADs from the normal diet were estimated to range from <1 to 600 µg/person/day (mean) and 23 to 3,599 µg/person/day (high-level). The intake of aloins from the analyzed Aloe vera products were below high-background HAD intakes in all instances, and only one sample exceeded the mean estimated exposure from all survey datasets, demonstrating that dietary exposure to aloins A and B from drinkable Aloe vera products are within the range of intakes of HADs consumed as part of the background diet. Furthermore, exposure to aloins A and B from the analyzed Aloe vera products is considerably lower than the exposure levels from supplements used for laxative effects reported by EFSA, of 1.2 to 31 mg/person/day.

1888 The Norwegian Biomonitoring Study in EuroMix: Real-Life Exposure to Bisphenols, Parabens and Triclosan in Humans as Measured in 24-Hour Urine Samples and Associations with Food Consumption and Cosmetics Use

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The Horizon 2020 EuroMix project develops validated test strategies for the hazard and exposure assessments of chemical mixtures. A human biomonitoring study was performed to study exposure to chemicals as present in foods and personal care products (PCPs). For two 24-h study periods separated by 2-3 weeks, adult volunteers (44 males and 100 females) in Norway kept detailed diaries on food consumption (type/brand, weight, packaging material and time) and the usage of personal care products (PCPs) (type/brand of product, time and number of applications, and number of showers and hand washes). Participants also registered the number of thermal paper receipts handled. Twenty-four h urine samples were collected. Bisphenol A (BPA), bisphenol S (BPS), bisphenol F (BPF), bisphenol AF (BPAF), parabens, triclosan (TCS), triclocarban (TRCB) and oxybenzone (OXB) were measured in the urine collected on the first day. Log transformed concentrations in urine were used in a multivariate linear regression (MLR) analysis with the main food and PCP categories. BPA was detected above the Limit of Detection in 96%, BPS in 29% and BPF in 4% of the urine samples. OXB, TCS, methylparaben (MePA) and ethylparaben (EtPA) were detected in 90-100% of the urine samples and propylparaben (PrPA) and butylparaben (BuPA) in 65% and 50%, respectively. The fraction of standard deviation used to characterize the mixture of chemicals was demonstrated. BPP, BPAF and TRCB were not detected. Higher paraben concentrations were measured in the urine of females compared to males, with mean concentrations of methylparaben (non-significant) and propylparaben (Pr/PA, P<0.05) of 108.16 and 8.82 ng/ml in females and 10.64 and 0.59 ng/ml in males. MLR analysis (all possible categories) showed a significant positive association between MePA and meat consumption (β=1.90, P<0.02), BuPA and bread consumption (β=1.78, P<0.004) and TCS and butter/oil consumption (β=2.12, P<0.002). With respect to the use of PCPs, a positive association was found in men between MePA and the use of hair conditioner (β=2.45, P<0.03) and hair styling (β=2.23, P<0.03), and TRCS and the use of shampoo (β=3.22, P<0.01). No significant associations were found between urinary phenols in females and the use of PCPs. Aggregated exposure estimates from both diet and PCPs are needed to explore the determinants from foods and PCPs on phenols exposure further.

1889 Derivation of Dermal Absorption Values Using Operator Exposure Biomonitoring Studies: A Case Study with MCPA

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Dermal absorption values were derived based on a biomonitoring study (where internal systemic exposures were determined) and the EFSA Model (giving predicted external exposures). The biomonitoring study measured operator exposures associated with the use of a MCPA formulation. Systemic (internal, actual) exposure to individual operators based on urinary excretion of biomarkers, MCPA and H-MCPA (4-chloro-2-hydroxymethyl phenosaccharic acid) was measured. The EFSA Model, adjusted for the specific exposure scenario parameters (application rate, water volume, area treated, body weight and clothing and PPE used), was used to estimate external exposure to these operators based on the exposure scenarios described for each operator in the biomonitoring study. The normal absorption values based on each operator based on the ratio of measured internal systemic exposure to predicted external exposure. A range of absorption values were derived. Following EFSA Dermal Absorption Guidance the mean dermal absorption value was corrected by adding a fraction of the standard deviation to derive a conservative value. The normal absorption values and the EFSA model were applied to the sample size, i.e. number of operators. Taking this approach ensured that the predicted external exposures reflected the measured internal exposures and, therefore, allowed a relevant, but health protective, exposure ratio to be determined and a dermal absorption value to be calculated as 2.9%. The pre-determined data established dermal absorption values of 2.2% (concentrate) and 2.5% (spray dilution) for MCPA which were based on in vitro human and rat in vivo rat studies performed on a different formulation containing MCPA (MCPA SANCO/4062/2001-final, 11 July 2008). In this case study MCPA dermal absorption values calculated using biomonitoring study data match with the dermal absorption values derived from dermal absorption studies. The derived dermal absorption value was then used to assess exposure for operators, residents and re-entry workers associated with handheld applications. All predicted exposures were below the reference dose, AOEL.

1890 Risk Assessment of the Flame Retardant Chemical Tris (1, 3-Dichloro-2-Propyl) Phosphate (TDCPP)

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TDCPP (13674-87-8) is a flame retardant that may be used in consumer products, such as upholstered furniture, to reduce fire hazards. TDCPP causes both cancer and non-cancer effects in rats (NRC 2000). CPSC previously established the acceptable daily intake of TDCPP as 5 µg/kg/d, and the unit cancer risk as 0.0031 (mg/kg/d)1. Recently, the National Health and Nutrition Examination Survey (NHANES) published 2013–2014 human biomonitoring (HBM) data on urinary concentrations of bis(1, 3-dichloro-2-propyl) phosphate (BDCCP), a major metabolite of TDCPP; HBM data can be used to estimate total human exposure. In addition, CPSC staff reviewed published data on TDCPP levels in household dust [1 to 6 µg/g] and indoor air (average 0.59 ng/ml); these data can be used to estimate TDCPP exposure from multiple sources in the home. In this study, we assess the health risks of total TDCPP exposure from NHANES data and the contribution of the home environment (dust and indoor air) to total risk. Total exposure was estimated from HBM data using a published fractional urinary excretion (FUE) value. We estimate the total average daily exposure (ADE) to be 34 ng/kg-d for adults and 107 ng/kg-d for children ages 6 to 11. This results in an estimated hazard index (HI) of 0.01 for adults and 0.02 for children, for non-cancer effects. The individual lifetime cancer risk is estimated to be 1.5 per million (average exposure) and 12 per million (95th percentile). Thus, some individuals exceed the level of concern for cancer risks of 1 per million. We also estimated the ADE from household dust and indoor air to be 1.2 ng/kg-d for adults and 5.8 ng/kg-d for children. Exposure from household dust and indoor air contribute a fraction (3% to 6%) of the total TDCPP exposure. Other exposure sources, such as direct contact with consumer products (e.g., furniture, baby products) and environmental exposures, should be considered in future risk assessments. This work has not been reviewed or approved by, and does not necessarily represent the views of, the Commission.
Biomarker Based Occupational Exposome

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Occupational exposures are thought to be responsible for over 370,000 premature deaths each year. Thus, the precise quantification of occupational exposure and associated adverse health outcomes is necessary to consider in understanding an individual’s exposome, the entirety of chemical exposures in a lifetime. Using the National Health and Nutrition Examination Survey (NHANES) of 116 chemical biomarker concentrations and 10 physiological stress response (PSR) biomarkers measured in 23,002 participants, we implemented an integrative approach to systematically contrast occupational exposure differences across 13 occupational groupings. We conducted a series of multiple regression models with chemical biomarker levels as the outcome variable and occupational groupings as the main predictor while adjusting for age, race, gender, study year, smoking, and poverty-income ratio. In addition, we conducted another series of regression models with each PSR biomarker as the outcome variable and occupational groupings as the main predictor while adjusting for the previously mentioned covariates with the exception of study year. We identified widespread biomarker levels across the different occupational groupings. For instance, biomarker levels of parabens in White Collars on average are higher compared to all other occupational groupings by fold differences ranging from 0.64 to 2.21. We have identified that on average biomarker levels of phthalates in workers from Accommodation, Food Services, and Construction are higher than those of White Collars by fold differences ranging from 0.96 to 1.53 for workers in Accommodation and Food Services and 0.93 to 1.25 for Construction workers. DEET as a metabolite of DEET, was observed to be of higher concentrations in Blue Collars in Mining compared to the White Collars by a fold difference of 3.88. C-Reactive Proteins, one of the PSR biomarkers, have been linked to chronic diseases and in our analyses show the most variability across the occupational groupings with the highest difference found when comparing Blue Collars workers in Mining with the White Collars (1.65 times higher compared to White Collars). Systematically studying the NHANES chemical biomarkers, physiological responses, and occupational data by implementing an untargeted approach enables the identification of expected and unexpected differences in exposure and health outcomes by job categories. These findings can be utilized to prioritize chemicals and workers for toxicological evaluation or health interventions.

Optimization of Real-Time Cell Metabolism Analyzer for Measuring Oxygen Consumption Rate (OCR) in Zebrafish Embryos Exposed to OPFRs

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Mitochondrial dysfunction is related to the occurrence of serious metabolic disease like diabetes, cancer and Parkinson’s disease. Inhibited oxygen consumption rate (OCR) is valuable evidence for mitochondrial dysfunction. OCR can be measured by real-time cell metabolism analyzer named Seahorse XF Extracellular Flux Analyzer. Recently, the attempt to apply XF-24 analyzer into the zebrafish embryo model is being conducted. However, the experimental conditions and interpretation of obtained results are not well standardized. In this study, we examined various factors (i.e. number of embryos, inhibitor concentrations, and fertilized time) to yield the most stable typical OCR tendency in XF-24 analyzer. The optimized factors were employed in zebrafish embryos exposed to sublethal concentrations (0 control), 3.45, 6.90, 13.80 μM (0.1% DMSO)) of Triclosan to confirm their applicability and exposed to Tris (1,3-dichloro-2-propyl) phosphate (TDCPP) and Triphenyl phosphate (TPH) (0 control), 0.0064, 0.064, 0.64 μM (0.1% DMSO)) to investigate the adverse effects of TDCPP and TPH in zebrafish embryos. The concentrations of three inhibitors were determined through 6 times of optimization experiments. Inhibitors’ concentrations for obtaining typical OCR pattern are followed; Oligomycin (Sigma + Kit) 12.5 μM, FCCP (Sigma + Kit) 8 μM, Rotenone/antimycin-A 1.5 μM. These concentrations were suitable for 3 embryos per one well. OCR exposed to higher concentrations of Triclosan (6.90, 13.80 μM) showed increased proton leak and decreased ATP-linked respiration in a dose-dependent manner. Exposure of TDCPP and TPH caused increased maximal respiration. This study suggests the determinants to obtain the stable OCR pattern in zebrafish embryos using the instrument of Seahorse XF-24 Extracellular Flux Analyzer, making it easier to analyze the comprehensive mitochondrial dysfunction in zebrafish embryos exposed to toxic chemicals of interest.

In Vivo to In Vitro Translation of Salivary Concentrations for Non-invasive Biomonitoring of 2,4-Dichlorophenoxyacetic Acid (2,4-D)

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Biomonitoring practices generally examine blood and urine to acquire insight regarding chemical exposures. Saliva has become a favorable sample matrix due to its non-invasive attributes and overall flexibility in collection. Relating concentrations measured in saliva to concentrations in blood or urine can be challenging due to gaps in knowledge of mechanisms driving salivary transport. We employed a Transwell in vitro system under physiological conditions to monitor and measure salivary chemical transport of a commonly applied herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D). High levels of protein binding (> 92%) were observed, resulting in transport (primarily via passive diffusion) to the salivary compartment limited by formation of free unbound species, predicting a saliva/plasma ratio of 0.034. Comparatively, a saliva/plasma ratio of 0.0079 was produced from an in vivo study, orally dosing male Sprague-Dawley rats with 30-150 mg/kg 2,4-D and collecting plasma and saliva 1 hour post-exposure. A physiologically based pharmacokinetic (PBPK) model was developed to translate observations from the cell culture model to those in animal models. The PBPK model notably incorporated descriptions of protein binding, salivary/plasma partitioning, and permeation. While apparent differences in in vitro and in vivo saliva/plasma ratios (0.034 and 0.0079, respectively) were observed, simulations with the PBPK model demonstrated dynamic saliva/plasma ratios over time and concordance between in vitro and in vivo experiments. This indicated that 2,4-D exhibits diffusion limited transport to saliva in vivo, with saliva/plasma ratios requiring longer time after exposure to reach equilibrium, compared to other small organic chemicals like trichloropyridinol. Sampling time after exposures is then a critical component for interpreting salivary 2,4-D biomonitoring data. This work demonstrates the potential of PBPK modeling and linking observations made with in vitro and in vivo approaches and additionally allows for further extrapolation of salivary concentrations for human biomonitoring. Supported by CDC/NIOSH grant R01 OH011023.

Exposure Assessment of Milk Protein in Non-dairy or Vegan Ice Cream Substitutes: Are Non-dairy or Vegan Products Safe to Populations with Milk Allergy?


Milk is recognized as one of the eight most common food allergens in humans in the United States, and is one of the most common causes of fatal or near-fatal food-induced anaphylactic reactions in adults and young children globally. Undeclared allergens in food products are the leading cause of US FDA requests for food recalls, with undeclared milk as the most frequently cited allergen. For example, a recent study by the US FDA found that dark chocolate bars frequently contained undeclared milk. The purpose of this study was to evaluate products marketed as non-dairy to investigate this problem and understand potential risk to allergic individuals consuming these products. In this study, milk allergen contamination in ice cream substitutes labeled as “non-dairy,” “dairy free,” and/or “vegan” was measured, including plain ice creams and those containing swirls and/or solid additives. Results from this analysis are used to determine if consumers of the ice cream substitutes poses a risk to allergic individuals by comparing the measured allergen concentrations to published threshold doses for a reaction to milk protein. More than 30 flavors of non-dairy and/or vegan ice cream substitutes, including plain flavors and flavors with swirl and/or solid additives, from 16 different brands were tested for total milk protein using enzyme-linked immunosorbent assay. Milk protein was detected in three of the tested flavors; two contained solid additives and one was a plain flavor. One of the three products with detectable milk allergen was labeled that it was manufactured on equipment or in a facility that processed milk. Based on the package serving size, one serving of some of the tested non-dairy ice cream substitutes could have several milligrams of milk allergen. There is no consensus on the minimal dose of milk protein that will elicit an adverse effect in a milk-sensitized individual, as the eliciting dose has variable estimates, depending on the population and metrics used. The US FDA reported that the lowest dose of milk protein associated with an observable adverse effect among milk-allergic individuals is approximately 0.3-3.6 mg. These results suggest that a single serving of some ice cream substitutes has the potential for an allergic response in a highly milk-sensitive individual.
1895 Chemical Characterization of Cigarette Smoke and Aerosol of the Candidate-Modified Risk Tobacco Product, Tobacco Heating System 2.2, Trapped in Biocompatible Solvent Using a Non-targeted Screening Approach


It is estimated that there are more than 6,000 chemicals in cigarette smoke (CS), many of which have been classified as harmful and potentially harmful constituents (HPHC) and are associated with smoking-related diseases. The Tobacco Heating System (THS 2.2) is a smoke-free product designed to heat tobacco rather than burn it. As a result, its aerosol is mainly composed of water, glycercin, and nicotine, with significantly lower levels of HPHCs compared with CS. Aqueous extracts (AE) are biocompatible fractions usually tested in vitro for product assessment. In this study, the chemical composition of both CS and THS 2.2 aerosol trapped fractions were investigated using a non-targeted screening (NTS) approach. AEs were generated by bubbling CS or THS 2.2 aerosol through an impinger containing phosphate-buffered saline. The applied NTS approach combining liquid chromatography and 2D gas chromatography coupled to mass spectrometry to comprehensively cover the chemical space of both AEs. The trapped constituents were identified according to a confidence match factor substantiated by complementary parameters, such as retention time, mass spectrum similarity, and library proposal. Their concentrations were measured as semi-quantitative values using stable isotopically labelled internal standard and with a 100 ng/item cut-off. In both aqueous fractions, 108 compounds were found with significantly lower concentrations in the THS 2.2 fraction compared with CS. About 70% of them were identified and confirmed by reference standards. The remaining portion of constituents were identified with either high or medium confidence, and only 3% were given as not identified with concentrations lower than 100 ng/stick. In conclusion, we found different chemical composition profiles between AEs derived from CS and THS 2.2 aerosol. Most of the constituents were identified and either confirmed by reference standards or providing high confidence in the proposed structures. Therefore, a good understanding of exposed system for in vitro product assessment was provided by the characterization of biocompatible fractions.

1896 Evaluation of Lead Exposure by Hand Wipes: Efficacy of Personal Hygiene on Industrial Sites

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It has been 25 years since the passage of the OSHA lead in construction standard (29 CFR 1926.62) meant to control both inhalation and ingestion exposures. Despite advances in engineering controls and work practices, elevated blood lead levels persist in the industrial painting industry. The purpose of this study was to evaluate the effectiveness of worker’s personal hygiene practices at removing lead from their hands at industrial sites (n=30). Hand wipe samples collected from three bridge painting projects were analyzed for total lead by method ASTM E-1979-17/EPA SW846 7000B. Exposures resulted from the removal of lead-based paint from the structure and trace elements were evaluated (n=6). Sixty unique hand wipe samples were evaluated. Thirty samples were collected during and after the work shift. These data will be useful in raising awareness in the industrial painting industry that personal hygiene practices must be improved to prevent the uptake of lead during the removal of lead-based paint.

1897 Biomonitoring of Polycyclic Aromatic Hydrocarbons in Breast Milk of Texas Women

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Biomonitoring of human breast milk is one of the best ways to identify body burdens of contaminants and associated risk estimation. The objectives of the current study were to evaluate milk concentrations of persistent organic pollutants, mainly polycyclic aromatic hydrocarbons (PAHs), associated exposure estimation, and the role of body mass index (BMI) in their bioaccumulation. A total of 45 breast milk samples were collected from 24 women with BMI ≥ 30 (obese) and 21 women with BMI < 25 (18.5-24.9, normal) from different counties in West Texas; age ranged from 18-34 years. Samples were analyzed using high resolution gas chromatography coupled with mass spectrometry (GC-MS). A total of 69% of samples tested positive for PAHs. Phenanthrene was the most frequently detected PAH following by pyrene and fluoranthene. The mean of individual PAH concentration for all samples ranged from 0.02 to 5.01 ng/mL; the sum of all means of individual PAHs was 29.38 ng/mL. The mean concentration of total PAHs in the BMI ≥ 30 group was 44.95 ng/mL, which was approximately 4 times the mean of total PAHs in the BMI < 25 group (11.58 ng/mL). None of the samples from the BMI < 25 group contained higher molecular weight (5-6 rings) PAHs, while in the BMI ≥ 30 group, a total of 11 PAHs listed as US EPA priority pollutants were observed. In this study, benzo[a]fluoranthene was found to contribute the highest percentage of carcinogenic PAHs (32%), yet it was not detected in any samples from the BMI < 25 group. These findings suggest that breastfeeding babies from obese mothers of West Texas are at higher risk of exposure to carcinogenic/obesogenic PAHs. However, babies fed with breast milk from lean body weight women are at relatively less risk of exposure to carcinogenic/obesogenic PAHs.
Human-impacted surface, ground and drinking water can contain a complex mixture of micropollutants, such as pharmaceuticals, pesticides, and industrial compounds. In vitro bioassays based on various cellular response pathways have been applied to detect and quantify the presence of micropollutants in water samples. These bioassays allow for sensitive, rapid and inexpensive detection of bioactivity while considering complex mixtures of compounds and potential toxicity. However, the current approach where single response pathways are examined sequentially limits its usefulness since it requires dozens of different cell lines and reporter systems and often does not cover a wide range of activities. In these studies, we developed a work-flow to maximize the number of receptors and pathways examined with enhanced throughput and toxicologic information. First, the water sample extract is examined in mixtures of optimized reporter assays that are grouped based on biological niche including: Xenobiotic and Bile Acid Metabolism (XM Panel; CAR2, CAR3, PXR, FXR, VDR, AhR), Lipid and Energy Metabolism (LEM Panel; THRB, PPARA, PPARB, RXRA, RXR, LXR), Reproductive and Developmental Effects (EDC Panel; AR, ERA, ERB, PGR, GR, TRH); Central Nervous System and Basal Metabolism (BM Panel; LRX, RXRB, RXRG, MR, RARG) and Toxicology and Inflammatory Pathways (TOX Panel; NFKB, NFR2, AP1, p33, MTP). Extracts that exhibit positive activity in one of these five panels (representing 28 pathways), can be examined in a dose-response to determine Bioanalytical Equivalents (BAEs) for that pathway. In addition, each receptor in the impacted panel can be explored individually to determine its contribution to overall activity. Several environmental and waste water samples and known contaminants of concern (CECs) have been examined in this manner.

**Assessing External Exposure to Chemical Mixtures Starting from Human Biomonitoring Data with the INTEGRA Computational Platform**

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The current study aimed at the estimation of external and target tissue exposure to a mixture of 15 different chemicals commonly found in human biomonitoring (HBM) samples, including both rapidly (BPA, DEHP, triclosan) and non-rapidly (PCBs, BDEs, HCB, DDT) metabolized compounds, starting from HBM data. The simulations were carried out on INTEGRA, a computational platform that provides exposure assessment coupled with a genomic physiology-based bio-kinetic (PBBK) model and numerical “reverse dosimetry” techniques (based on Markov chain Monte Carlo and dynamic evolution Monte Carlo techniques) for exposure reconstruction. The process starts from ancillary exposure-related data that are fed into the exposure model. The results are evaluated against the HBM data distributions, aiming at the reduction of uncertainty in back-calculating doses, by minimizing the error between the predicted and the actual HBM data. Parameterization of the model for a large number of receptors and pathways are examined sequentially limits its usefulness since it cover a wide range of activities. In these studies, we developed a work-flow to maximize the number of receptors and pathways examined with enhanced throughput and toxicologic information. First, the water sample extract is examined in mixtures of optimized reporter assays that are grouped based on biological niche including: Xenobiotic and Bile Acid Metabolism (XM Panel; CAR2, CAR3, PXR, FXR, VDR, AhR), Lipid and Energy Metabolism (LEM Panel; THRB, PPARA, PPARB, RXRA, RXR, LXR), Reproductive and Developmental Effects (EDC Panel; AR, ERA, ERB, PGR, GR, TRH); Central Nervous System and Basal Metabolism (BM Panel; LRX, RXRB, RXRG, MR, RARG) and Toxicology and Inflammatory Pathways (TOX Panel; NFKB, NFR2, AP1, p33, MTP). Extracts that exhibit positive activity in one of these five panels (representing 28 pathways), can be examined in a dose-response to determine Bioanalytical Equivalents (BAEs) for that pathway. In addition, each receptor in the impacted panel can be explored individually to determine its contribu
1903 Improving Agrochemical Risk Assessment: Building Confidence in Estimating Chronic Dietary Exposure at Early Stages

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Within the human health assessment paradigm, exposure assessment is a core component for assessing and preventing potential adverse human health outcomes. In current agrochemical risk assessment, extensive and multiple-year residue trial studies are required but usually not available for estimating human chronic exposures until later stages of new agrochemical development. This is a problem in the new era of problem formulation, human exposure-based toxicity testing and Risk21-like approaches. Therefore, a platform was established to predict chronic exposures at early stage by integrating modified DynamICROP model (for predicting residue levels in various crops) and Dietary Exposure Evaluation Model (DEEM, for predicting human dietary exposure) using very limited residue trials. A case study demonstrated that the predicted exposures were within 5% relative to that from measured residues for all subpopulations. However, for all active substances, following initial product registrations, many changes (such as type and number of formulations, application rates, application methods as well as expanded crops) can be made resulting in changes in potential human exposure. To understand the exposure change patterns during the course of post-launching and implication of the predicted exposure from the Platform, a retrospective study on human chronic dietary exposures to 37 active substances across 30 years was conducted by comparing the first and last available exposure data obtained from public available risk assessment documents. For substances with no drinking-water assessment, DEEM was conducted. To calculate water exposure based on maximum estimated concentrations in drinking-water which was then combined with exposure from food. In general, the results indicate that 97% of active substances including herbicides, fungicides and insecticides have less than 10-fold (1-8-fold) change to general and the most sensitive populations over this period on the market. Based on these results, in the absence of future product use pattern changes, chronic exposures can be reasonably predicted using the Platform and an extrapolation factor of 10 to represent possible future worst case human exposures. The confidence in exposure estimates at the early stages can provide a basis for exposure-driven toxicity testing as well as tiered toxicity acquisition to inform internal and potentially regulatory decision-making.

1904 Post-Hurricane Harvey Sediments: PAH Distributions and Relative Sources

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In the fall of 2017, Hurricane Harvey was responsible for severe flooding all over Houston, TX and there is a need to understand the distribution of contaminants within the impacted region. The first analyte class to be extensively covered for most of the sediment samples collected post-Harvey are polymeric aromatic hydrocarbons (PAHs). They are ubiquitous compounds in the environment, originating from petroleum as well as incomplete combustion and the pyrolysis of organic matter. What is unique for Houston, is the diverse source inputs for these compounds and the ever changing dynamics of the region. As a result of the severe flooding, sediment beds in the northern parts of the Houston estuary were shifted and moved into Galveston Bay (GB). Therefore, we hypothesized there is a new baseline for any contaminants present in soil and sediment samples. As a part of collaborative efforts with the School of Public Health and TAMU Galveston, soils and sediment samples were collected over the course of Fall 2018 where they have been analyzed for a detailed suite of analytes; which include: PAHs, Organochlorine Pesticides, Polychlorinated Biphenyls, and Dioxins/Furans. For the sediment samples, PAHs were the first analyte analyzed, where appropriate ratios indicate most of the sources are pyrogenic in nature; such as vehicular emissions. PAH totals range from 152.3 µg/kg to 4291.5 µg/kg with initial spatial analysis indicating high spatial dependence amongst the samples collected. Based on these results, we are in the process of developing a geospatial sampling map for future analyses utilizing statistical predictive methods. From these the initial PAH maps, local and regional stakeholders will be equipped with an understanding of potential exposure routes of any detected PAHs. This research was funded by P42 ES027704 and T32 ES026568.

1905 Perfluoroalkyl Acid Mixtures—Data Analysis Steps to Uncover Clues Hidden in Biomonitoring Data

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Biomonitoring programs serve a critical role in guiding strategies to identify and manage chemical exposures that may present a risk to public health. Well-designed biomonitoring programs can provide critical information to address questions about the relationships between exposures, toxicokinetics, and health outcomes, particularly in the case of classes of chemicals that tend to occur as mixtures. Using data reported on populations exposed to perfluoroalkyl acids (PFAs) in drinking water, we demonstrated a bio- kinetic slope factor that properly accounts for background exposure, which can be estimated from the National Health and Nutrition Examination Survey (NHANES). Using perfluorooctanoic acid (PFOA) and perfluoromonoamino acid (PFNA) as a case study, we illustrate how current approaches based on ratios of concentrations in drinking water and serum yield estimates of the biokinetic slope factor that are biased high by a factor of 2 to 5 because they reflect the combination of baseline and drinking water, rather than drinking water alone. We provide a side-by-side assessment of key studies that inform current kinetic models, and highlight examples of how variability in species-specific kinetics and exposure sources impact interpretations of biomonitoring data. Specifically, we use first-order kinetics models to quantify the inherent interindividual-variability in volume of distribution, and corresponding estimates of serum elimination half-life and clearance, and we explain how this informs the interpretation of the observed range in serum levels for a community exposed to a range of drinking water concentrations. Furthermore, we show how uncertainty in estimates of the relative serum half-lives of PFOA and PFNA can be resolved by examining the PFAA mixtures paired at the individual level. Applying these data analysis steps to biomonitoring data in communities with known drinking water contamination can improve estimates of the time course of changes in PFAS both before and after intervention.

1906 Indirect Dietary Exposure Assessment of Surface Cleaner Residues (ADBAC) and Refinement Using a Novel Analytical Residue Method

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Alkyl dimethyl benzyl ammonium chlorides (ADBAC) encompass a cluster of 24 structurally-related quaternary ammonium compounds (QAC). Typically, these organic salts only differ with regard to alkyl chain lengths and are often commercially available in biocidal disinfectants/antiseptic products. Moreover, ADBAC has a ubiquitous presence in consumer goods: ranging from personal care to surface cleaners. In the evaluation of product safety, exposure-based risk assessment is a necessity - not only as it pertains to product use but also in regard to the unseen residues on residential food-contact surfaces. Generally, in the absence of data, worst-case exposure assessment is conducted. However, the result is often an overly conservative, unrealis- tic estimation of surface residues. Therefore, a novel analytical method was developed and validated which allows for a near-complete collection and accurate quantification of residues using a variety of real-world product-types (wipe, aerosol, trigger spray), cleaning substrates (paper towels, sponges, cloths), and cleaning surface (stainless steel, formica, ceramic). Collectively, these potential variables were evaluated in a number of experiments spanning several Latin-square matrices to decipher the most influential factors which contribute to ADBAC surface residues. Ultimately, we found that both product and surface type were significant in dictating total residual ADBAC concentrations and relative percentages respectively; whereas cleaning material was an insignificant determinant. Honing-in on these two factors, tier-based exposure assessment in line with US EPA practice, comprised of Tier 1 (A-Default assumptions of FDA EDI methodologies, B-refined based on transfer general efficiency, C-refined using measured surface residues), Tier 2 (IDREAM - food consumption model using default parameters); and Tier 3 approaches (IDREAM - refined using chemical-specific residue values). Ultimately, we found that even at Tier 1 (worst-case scenario), the corresponding ADBAC exposures were below US EPA established Chronic Population Adjusted Doses. However, refinement from Tier 1 to Tier 3 exposure assessment resulted in a significant reduction of estimated doses, more interestingly we found that these de- clines were disproportionate and dependent on product type (Reductions: Aerosol >7-fold; Trigger >27-fold; Wipe >100-fold). The findings of the study are important in both claims substantiation and safety evaluation of cleaning products positioned for use on food contact surfaces.
In commercial and academic facilities, various additive and subtractive manufacturing technologies such as 3D printing and laser cutting are used. These processes are associated with emission of volatile organic compounds (VOCs) and particle pollution depending on the specific activity. The sampling site selected in this study was a Makerspace facility with a variety of machines including 3D printers (Enclosed and Open) and Laser Cutters. The emission of ultrafine particles (UFPs) and VOCs, from each type of these machines, was quantified and the input/contribution of each machine in the indoor air quality was estimated. Ultrafine particles were measured using a TSI P-trak. VOCs were measured with a real time monitor with a PID sensor (MOCON Inc.) and grab samples were taken using evacuated canisters and analyzed by GC-MS. For an enclosed 3D printer using ABS filament feedstock, a comparison of the front and rear emissions showed higher UFPs at the rear (3000-5000 pt/cm³) at the beginning of printing and going down to a background level of 1383 pt/cm³, while VOC emissions were higher at the front with the real time moni- tor peaking at 700 ppb in the middle of printing. Canister samples showed increases in acetone, ethanol, methyl methacrylate, and styrene during the 3D printing process. With an open 3D printer using PLA filament, VOC and UFP emissions surprisingly remained at the same levels as the background concentrations. Sampling at a laser cutting acrylic plastic showed higher total VOC emissions in front of the machine when the lid was opened right after the job completion, with a peak reading of 2251 ppb. In the grab sample, the concentration of methyl methacrylate and methylene chloride went up to 51.7 and 3.4 ppb compared to a background of 0 ppb. Interestingly, sampling at the exhaust assembly for the laser cutter showed a maximum increase of these two analytes 4.4 and 5.1 times greater than the concentrations observed in the front of the machine. UFP numbers peaked at 16000 pt/cm³ when the laser cutter lid was opened after completion of cutting with a mean (SD) concentration over the entire period of cutting of 2376±591.8 pt/cm³. In conclusion, emissions from burgeoning manufacturing technologies contributed to the airborne concentrations of volatile gases and particle in the Makerspace facility, but the levels observed in this study appeared to remain within recommended occupational exposure limits.

Flumetralin, a substance with plant growth regulating activity, is applied as a topical treatment for the control of sucker growth on various types of tobacco. The dissipation and residues of flumetralin 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 78-96% with relative standard deviations (RSD) less than 15%. The limit of detection (LOD) and limit of quantification (LOQ) of flumetralin was 0.9 and 3 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 flumetralin appeared to follow the first-order kinetic reaction with half-lives of 3.5-5.8 days at four geographical experimental plots, which suggested that the dissipation of flumetralin in the field might be affected by some physical and chemical factors like rainfall, light, heat and moisture in addition to growth dilution factor. Tobacco flue-curing experiments were carried out after the field trials following the three-stage curing technology. Tobacco leaves collected from final residue experiments were processed. About 56.7-96.3% of the residues had degraded after tobacco flue-curing, it suggested that high temperature during tobacco processing seemed to be the most important factor affecting degradation. The terminal residues of flumetralin at two dosage were less than 4.62 mg/kg in any locations, which never exceed the guidance residue level (GRL) established by CORESTA (5 mg/kg). This study could provide guidance for the safe use of flumetralin and serve as a reference for the establishment of maximum residue limit (MRL) in China.

Effects of Glyphosate and Its Formulations on DNA Damage in HepaRG and HaCat Cell Lines

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Glyphosate (GLY) is an active ingredient found in herbicide formulations around the world. It affects the shikimate pathway in plants, blocking the activity of 5-enolpyruvylshikimic-3-phosphate synthase (EPSPS). First reported in the late 1970s, but intensive use of GLY began with the introduction of GLY-resistant crops in the late 1990s. Conflicting reports exist as to whether GLY poses a cancer risk to humans, though it has a low toxicity profile for humans and mammals. Both European regulatory agencies and the US EPA have described GLY as unlikely to pose a carcinogenic hazard to humans, but the
International Agency for Research on Cancer (IARC) 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 tested GLY in two human cell lines, HepaRG (metabolically competent hepatocytes) and HaCaT (human keratinocytes) using the γ-H2AX assay which detects DNA double strand damage. Thirteen formulations with high concentrations ranging from 3 mM to 103 mM and 5 actives including GLY, GLY salts, and other active ingredients in the formulations were tested at ten different concentrations post 1- and 24-hour exposure. The results were then compared with the effects of glyphosate and its formulations on cytotoxicity (CellTiter-Glo assay) to determine whether GLY induces oxidative stress and DNA damage. While the positive controls etoposide and diquat elicited a markedly increase in γ-H2AX phosphorylation at concentrations lower than those that reduced cell viability, glyphosate and its formulations showed no or minimal γ-H2AX phosphorylation, and then only at cytotoxic concentrations that induced significant cell viability. In fact, the only formulation to show some DNA damage contains diquat as an active ingredient. This data suggests that GLY does not induce double strand DNA damage in HaCaT and HepaRG cells. It should be noted that the formulations marginally increased oxidative stress only after significant loss of cell viability. The results were very similar in both the HepaRGS and HaCaT cells, suggesting that xenobiotic metabolism has negligible impact on toxicity.

**1912 Role of the Organic Backbone and Metal Moeities in Alteration of Essential Metal Levels after Oral Mancozeb Exposure in Sprague-Dawley Rats**

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Mancozeb (MZ) is a broad-spectrum fungicide of the ethylene bisdithiocarbamate (EBDC) class. Use in agriculture raises questions about the effects of oral exposure of MZ on consumers, pesticide manufacturers, and applicators. Previous studies have shown that oral exposure to MZ in rats results in increased Gl tract Mn and increased renal Cu levels, raising concern due to altered metal homeostasis. MZ is comprised of an EBDC backbone complexed to Mn and Zn metals. It is suggested that MZ dissociates to its components in the body. The aim of the present study compared the altered metal profiles of MZ-dosed rats with rats dosed with namb (NB) (sodium salt of EBDC backbone) or with metal moieties (Mn and Zn) alone. MZ by weight is 77.5% EBDC, 20% Mn, and 2.5% Zn. To give equivalent doses of the components in 100 mg/kg MZ the EBDC group was dosed with 95 mg/kg NB and the Mn/Zn group was given a combined dose of 46 mg/kg MnCl₂ and 5 mg/kg ZnCl₂ (to give 20 mg/kg Mn and 2.5 mg/kg Zn doses, respectively). Sprague-Dawley rats (n=32) were divided into 4 groups: 25% PEG400 (vehicle), NB (EBDC), Mn/ Zn (Mozieb), and MZ and dosed for 28 days. After the 28th dose, rats were fasted overnight. On 29th day organs were harvested from euthanized animals and frozen until analysis. Analyses of Mn, Zn, and Cu were obtained using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) and results were expressed as ppm metal per g dry organ weight (ppm/g). In kidney, NB and MZ treatment resulted in Cu increase (76.69 and 78.39 ppm/g) compared to controls (9.696 ppm/g). Livers of MZ rats showed a decrease in Mn levels (2.114 ppm/g) compared to control (0.3637 ppm/g). A significant increase in Cu (3.742 ppm/g) relative to control (4.132 ppm/g) was observed in myocardium of NB and MZ treatment rats. DS treated rats showed a significant increase in Cu levels (10.23 ± 0.55 ppm/g dw) vs control (5.44 ± 0.06 ppm/g dw) in the myocardium. No significant changes in metal levels were observed in the vatsus medialis of MZ or DS treated rats as compared to control. This study has shown that DTGs alter metal levels in only myocardium of Long-Evans rats, and may not have a direct influence on skeletal muscle damage. Additional research is being conducted to determine the mechanism of toxicity that DTGs cause on both myocardium and skeletal muscle.

**1914 Determination of Offspring NOAEL for Zeta-Cypermethrin from Developmental Neurotoxicity Studies in Rats Using Internal Exposure Data**

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Zeta-cypermethrin is an enriched isomer of cypermethrin, a synthetic pyrethroid. Range-finding and definitive dietary developmental neurotoxicity (DNT) studies have been conducted in the 1990s. A pharmacokinetic (PK) study in PND 11, 21 and 90 female rats was recently conducted to obtain data that would allow for a more accurate calculation of offspring NOAEL. In the definitive DNT study (dosages: 0, 50, 125 and 300 ppm; 0.3, 6.0, 9.0 and 21.1 mg/kg/day for dams during gestation; 0.8, 7.1, 41.4 and 45.7 mg/kg/day for dams during lactation), maternal body weight and food consumption, offspring body weight were reduced significantly at 300 ppm. Zeta-cypermethrin was not considered to be developmentally neurotoxic from the results showing no CNS domain-, age- or test article-related adverse pattern of DNT in the offspring. The NOAEL for maternal and offspring systemic toxicity was determined to be 125 ppm. Plasma concentrations were determined at a single time point, approximately 1 hour after the dark period which corresponded to expected peak concentrations following dietary consumption. The maternal/offspring plasma concentrations of zeta-cypermethrin were 0.535/0.245 mg/L (mean value on LDS and 21) during PNDs, 21. In the single gavage dose PK study, after 0.25 mg/kg, the C₀ values were 0.094, 0.140 and 0.037 mg/L, respectively; the AUCs were 0.804, 0.180 and 0.229 mg·h/mL, respectively, in PND11, 21 and 90 rats. At 0.75 mg/kg, the C₀ values were 0.344, 0.158 and 0.108 mg/L, respectively; the AUCs were 2.329, 1.210 and 0.779 mg·h/mL, respectively, in PND01, 21 and 30 rats. These data suggest that tₕ½ values in the PND 11 and 21 are about 3X and 2X higher than those in the PND0 rats at the same dose, and it required about 1/3 and 1/2 doses in the PND11 and 21 to produce the same levels of C₀ and AUC in PND0 rats. The dose normalized C₀ (the slope factor of the linear PK curve) is 3X and 2X higher in the PND1 and 21 than that in the PND90 rats. Therefore, by using the mean maternal/offspring plasma concentrations (0.535/0.245 mg/L), a more conservative 3X relative PK factor and the equation 35/20 mg=r2456 mg/kg of intracellular NOAEL (Cu) with the group is calculated to be 3.3 mg/kg which should be protective of all human populations including children.

**1915 An Investigation of the Antifungal Activity of Forty Ebselen Analogs in Candida albicans**

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The growing emergence of fluconazole as well as multidrug-resistant strains in immunocompromised patients justifies the need for novel antifungal targets. Our lab has previously described the antifungal mechanism of ebselen.
EB, an organoselenium compound showing growth inhibitory activity (IC50 ~15 µM) for various C. albicans strains. In view of the current interest in organoselenium inhibitors of the plasma membrane H+ -ATPase, the present study was undertaken to investigate the antifungal activity of forty novel EB analogs in S1 (fluconazole sensitive) and S2 (fluconazole resistant) strains. Cells of both strains were prepared in RPMI 1640 medium using the microliduction method and were incubated for 48 hr with each EB analog. Amongst the test compounds, three of them (G-4, G-13, and G-30) surpassed the antifungal activity of EB in both strains. To confirm the growth inhibitory effect in living yeast cells, we determined the minimum inhibitory concentration (MIC) for the three most active test compounds along with EB and fluconazole using a colorimetric assay based on conversion of resazurin dye in 96-well plates displayed a MIC of 25 µM in both S1 and S2 whereas fluconazole-treated wells reduced the dye to pink color at all concentrations (MIC >100 µM) confirming that it is a fungistatic agent. G-4 and G-13 exhibited MIC values of 25 µM and 12.5 µM, respectively, in the S1 strain and 25 µM for both compounds in the S2 strain; whereas G-30 demonstrated a MIC of 6.25 µM in S1 and 12.5 µM in S2. In addition, medium acidification assays were performed for 30 min with each of the three most active compounds along with EB and fluconazole. As anticipated, fluconazole showed no effect on medium acidification, whereas EB inhibited medium acidification (IC50 ~ 5 µM) in both strains. G-4 and G-13 showed a similar inhibitory effect as EB in a time- and concentration-dependent manner (IC50: 3.6 µM and 4.7 µM, respectively, in S1 yeast and 5.6 µM and 5.2 µM, respectively, in S2 yeast). Compound G-30 displayed no inhibitory effect for the first 15 min; however, inhibition of medium acidification was observed thereafter. We hypothesize that this late inhibitory effect on medium acidification may be due to the bulky nature of the test compound. Taken together, the present results indicate that modified EB analogs should be investigated further for use as antifungal agents in fluconazole-resistant as well as other C. albicans strains.

1919 Carboxylesterase Isoform-Selective Inactivation in Lung following Oral Chlorpyrifos Exposures of Neonatal and Adult Mice

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Chlorpyrifos (CPF) is an organophosphate (OP) pesticide known to exhibit toxicity via inhibition of acetylcholinesterase (AChE) in the nervous system. We previously showed that endocannabinoid (eCB) metabolizing enzymes were even more sensitive than AChE to inhibition by OP pesticides in neonatal rats, leading to increased levels of eCBs in brain. Because eCBs are known to be immunomodulatory molecules, we are investigating a link between eCB metabolism and immunity in adults and neonates exposed to CPF. We hypothesized that neonatal mice would be more sensitive than adult mice to the effects of CPF on eCB metabolism. Adult mice (≥ 8 weeks old) and neonatal mice (post-natal day 10) were treated with CPF (2.5 mg/kg oral) or vehicle daily for 7 days. Tissues were harvested 4 hr after final treatment. Lung microsomes from both age groups demonstrated marked inhibition of carboxylesterase (Ces) activity, one of the known eCB metabolizing enzymes. Lung Ces activity in neonates was more sensitive to CPF than adults. Activity-based protein profiling (ABPP) and immunoblotting of lung microsomes confirmed that Ces1 was present in both age groups, and the activity was inhibited by CPF. ABPP-mass spectrometry of lung microsomes identified 31 serine hydrolases in the neonatal lung and 32 serine hydrolases in the adult lung. Ces1d was selectively inactivated by CPF in neonatal lungs and Ces1c was selectively inactivated by CPF in adult lungs, indicating an age-related difference in sensitivity to CPF. In follow-up experiments in adult mice, treatment with WWL229, a selective Ces1d small-molecule inhibitor, potentiated lung IL-6, TNF-α, and IL-1β mRNA expression in response to lipopolysaccharide. Further studies will determine if treatment with CPF and LPS yield similar results, as this could indicate a mechanism by which low-dose CPF is immunotoxic to neonates. Moving forward, the overall goal of this project will be to explore age-related Ces1 sensitivities and the role of inhibition of Ces1d by CPF in pulmonary inflammation. Supported by MSU-CVM and R15GM128206.

1917 Genetics of the Effect of Paraquat on Iron, Copper, and Zinc in Mouse Midbrain

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Paraquat is an herbicide used widely in many countries, including the USA. It has also been implicated as a risk factor for development of sporadic Parkinson’s disease, especially in those living in agricultural areas and drinking well water. Although there is evidence for risk, not all epidemiological studies are consistent. The reason is likely genetically-based differential susceptibility to paraquat neurotoxicity in sub-populations. To address this issue, we have tested the effects of paraquat in a genetic reference population of mice (the BXD recombinant inbred strain family). In our earlier work, we showed that in genetically susceptible mice, paraquat increases iron in the ventral midbrain, the area containing the substantia nigra. Our hypothesis then is that the iron increase contains loosely-bound or unbound iron that in turn causes neuron toxicity by producing free oxygen radicals. To test the hypothesis more fully, we treated male mice from 28 of the BXD recombinant strains with 3 doses of paraquat, 1, 5 and 10 mg/kg three times on a weekly basis. At the end of the treatment period, we euthanized the animals and analyzed the ventral midbrain for concentrations of iron by total reflection x-ray fluorescence. Because our instrument (Bruker PicoFox) analyzes most elements, we also measured copper and zinc. The results showed that at 1 mg/kg compared to control, 2 strains showed a decrease in [Fe] and 7 showed an increase. For [Cu] 5 strains showed a decrease and 13 an increase. We also observed a significant association between a Ce marker and a genetic marker on chromosome 7 at 90 Mb. The search for a candidate gene at that location produced Picalcim, phosphatidylinositol binding clathrin assembly protein; intron 1. This gene is involved in iron regulation and is a one of the candidates for Alzheimer’s disease. At 5 and 10 mg/kg of paraquat, we also observed differential responses in increases and decreases of all three metals, at 1 and 5 mg/kg the concentrations of copper and zinc were correlated and at 10 mg/kg, iron and copper and copper and zinc were correlated. These results show that paraquat can affect the regulation of all three metals in the ventral midbrain and therefore one means of producing neurotoxicity.

1918 Cancer Risk Assessment for Agrochemicals: Are Non-cancer Endpoints Protective of Tumor Endpoints?

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Human health assessment of agrochemicals requires a comprehensive battery of toxicological studies covering different life stages and exposure durations. The carcinogenicity studies in both rats and mice are designed to assess tumorigenic potential of substances and have been implemented in the toxicity testing program for the past 5 decades. Given the significant animal use, it is important to consider, for non-genotoxic carcinogens (NGTxCs), if tumorigenicity must to be known in animals to protect again tumors for human health protection. A retrospective analysis on one hundred and twenty-eight (128) non-genotoxic agrochemicals was conducted to investigate (1) if tumor thresholds exist based on chronic NOAELs from the same study, (2) whether nonchronic non-cancer NOAELs with certain extrapolation factors (Efs) can be protective of cancer, and (3) if NOAELs from non-carcinogenicity studies (e.g. developmental or reproductive studies) being used to derive Acceptable Daily Intakes (ADIs) are more sensitive than tumor NOAELs. The results indicate that the tumor NOAELs are uniformly less or equally sensitive to chronic non-cancer NOAELs in both rodent species, indicating threshold effect for tumor endpoints. Based on the established correlation between rodent chronic and chronic NOAELs, when Efs of 6 and 5 are used to the rat and mouse subchronic NOAELs respectively, the predicted chronic NOAELs are protective of tumors except for 2 substances (sulfosulfuron in rats and forchlorfenuron in mice). Further investigations showed that sulfosulfuron induced tumors are due to irritations resulting from calcul and are generally not considered relevant for human risk assessment and the forchlorfenuron result is due to unusual dose spacing (tumor NOAEL and LOAEL are 4.3 and 500 mg/kg bw per day, respectively). For more than 30% (38/128) of ADIs derived from non-carcinogenicity studies, their NOAELs were exclusively lower than tumor NOAELs. Taken together, the results indicate that NGTxCs caused tumors in rodent thresholds do not exceed the accepted range of carcinogenicity studies, systemic rodent subchronic NOAELs with defined Efs or other more sensitive NOAELs are protective of tumor endpoints. These findings coupled with convincing evidence that non-cancer hazard can be adequately identified and protective points of departure can be established from other studies demonstrate limited utility of cancer bioassays in risk assessment.

1919 The Integrated 90-Day Rat Study Design: A Decade of Experience and Regulatory Acceptance

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The 90-day rat study is a fundamental toxicity study conducted within the package of studies defining the toxicity profile for agrochemical active ingredients. For a decade, it has been possible to integrate additional endpoints and obviate need for separate studies. Experience from 8 integrated 90-day rat studies, including study design, the need (or not) for additional studies.
examining the same endpoints and an overview of regulatory acceptance of this study type is presented. The study designs for eight 90-day rat studies with agrochemicals (sulfoxaflor, halaluxifen-methyl, halaluxifen-acid, florpyn-ixufen-benzyl, fenpicoxamid, XDE-469, XDE-607 and experimental fungicide) were compared. The base guideline was OECD 408, adapted to include OPPTS 870.7800 (immunotoxicity), OPPTS 870.6200 (neurotoxicity), and OECD 474 (mucinocular test). Integrated toxicokinetics (TK) measurements (blood/urine) were included with no additional animal use. Mode-of-action tissue samples were stored for potential toxicogenomic analysis. Results show 100% of these studies that were submitted to global Regulatory agencies (including EU, US, Canada, Australia, New Zealand, Brazil, China, India, and others) were accepted. The improved study design uses 130 animals in 12-232 as support for the separate studies. An analysis of each chemical was conducted to investigate which separate studies were not necessary using the integrated design. In 100% of these studies submitted, separate immunotoxicity and neurotoxicity studies have never been conducted or requested by any Regulatory agency. In 88% of cases, a separate OECD 474 was conducted as complementary to the data package. In 38% of cases, integrated TK data contributed to a kinetically-derived maximum dose (KMD) approach in follow-up studies. In 50% of cases, integrated MoA data was critical to the overall toxicity profile and avoided separate studies. In summary, in a 90-day rat study it’s possible to include immunotoxicity, neurotoxicity, genotoxicity, mode-of-action and TK endpoints whilst adhering to the guideline requirements. A recent example is the required in vivo MNT with recent guidance for demonstration of systemic exposure of the test compound such that integration in the 90-day study along with TK aids in the justification for dose selection and validity of the MNT results alongside assessment of systemic toxicity. These studies have been accepted in all geographies for new agrochemical registrations. This study design should be standard for agrochemicals and represent a significant 3Rs improvement.

1920 Evaluation of the Application of an AEP/AOP Framework to an Agrochemical, Sulfoxaflor
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The application of a combined Adverse Outcome Pathway (AOP) and Aggregate Exposure Pathway (AEP) framework to an insecticide was investigated. AOPs describe the Key Events (KEs) that follow a molecular initiating event (MIE, i.e. a binding to a receptor) and result in an adverse outcome. Sulfoxaflor (“Slocast”) is a sulfoximine insecticidal acetylcholinesterase (AChE) inhibitor. In rats, it induces developmental toxicity, mediated by the binding (MIE) and prolonged agonism (KE1) of the foetal-type muscle nAChR, leading to sustained muscle contraction (KE2), limb contracture (forelimb flexure and hindlimb rotation), clavicle abnormalities (from shoulder girdle contracture) and decreased neonatal survival (from diaphragm contracture, causing breathing abnormalities). In the application of the AOP approach, the critical doses causing effects, and/or the highest doses not causing effects, were identified for each of the KEs. The AEP framework allows for comparison and integration of exposure data to the specific AOP, in this case, sulfoxaflor mediated developmental toxicity, and considers available toxicokinetic (TK) data while potentially informing exposure and human health risk assessments. TK data were available for most of the key in vivo studies, enabling a qualitative comparison of effect levels between in vitro, ex vivo and in vivo studies in different species. This data enabled the AEP approach to be applied to sulfoxaflor at two levels: predicted external exposure of the test compound such that integration in the 90-day study design should be standard for agrochemicals and represent a significant 3Rs improvement.

1921 Hemp-Based Formulation Disrupts Development and Increases Mortality in Houseflies (Musca domestica)
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Musca domestica, otherwise known as the housefly, serves as a vector for over 100 human and animal diseases. Flies carry disease-carrying organisms in their mouthparts, body surfaces, feces, and vomit. The problem has been exacerbated by over spraying with toxic insecticides resulting in the development of insecticide resistance. As a strategy to control these pests without harming nontarget organisms, Fayetteville State University has developed a hemp-based formulation to specifically target flies. Previous studies found hemp to disrupt development in fruit flies; therefore, it was hypothesized that the hemp formulation would disrupt development in houseflies. Three main feeding assays were conducted: one to determine toxicity in the larvae, one to determine development, and one to determine developmental effects on the flies as they developed from egg to adult. The flies were subacutely exposed to the hemp formulation for up to nine days at the following concentrations: 0, 25, 50 and 100%. Findings showed significant increases in mortality for all stages tested. In the development assay, the larvae exposed to the hemp formulation died 2 days after the control group. Once the adults emerged, those exposed to the formulation convulsed and died within 2-3 days of emergence. In the adult toxicity assay all adults in the 100% concentration died within a 2-day exposure. It was concluded that the hemp formulation was effective in controlling adult flies and larvae. While adult flies exposed to the 100% concentration died within 2 days of exposure, development of larvae was disrupted with an exposure concentration of 50%. Future studies will include biochemical analyses to test acetylcholinesterase (AChE) levels in the flies at all stages. This enzyme could be a target site for the formulation and would explain convulsions in the adults. Analyzing hormone levels in the larvae may explain the occurrence of developmental defects since AChE may regulate ecysone or juvenile hormone levels, both of which control molting and metamorphosis in the larvae.

1922 Efficacy of Hemp Seed Formulations for the Control of Darkling Beetles (Alphitobius diaperinus) with No Observed Toxicity in Hens Following Surface Treatment of Bedding
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The darkling beetle (Alphitobius diaperinus) is one of the major pests of poultry houses causing millions of dollars of structural damage each year. In addition, darkling beetles also transmit disease in poultry. While synthetic pesticides such as imidacloprid and pyrethroids have been applied primarily to control this problem, concerns about toxic residues have prompted the need for safer control alternatives. The present study examined the efficacy of a patented hemp seed formulation for the control of darkling beetles after surface treatment of poultry bedding. In addition, the safety of hemp formulations on hens was determined and results were expressed as adverse effect levels of hemp formulations on their bedding for six weeks. Following exposure to the hemp seed formulation, darkling beetles laid significantly fewer eggs. Increased mortality was also observed in adult beetles following exposure to hemp seed formulations. However, no toxicity was observed in hens exposed to surface treatments. Specifically, no changes in body weight and number of eggs produced by hens were observed. In addition, no changes in liver toxicity indicators, such as aspartate aminotransferase and alanine aminotransferase, were observed. Findings suggest that the patented hemp seed formulation is an effective and safe alternative to control poultry pests.

1923 Detecting Toxic Effects of Roundup Concentrate Plus with the DEL Assay
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Sponsor: R. Schiestl

Pesticides have become a worldwide source of pollution and its ever-increasing use has attributed to detrimental health effects over the past decade. About 1 billion pounds of pesticides are used each year in the United States and among human health risks, exposure to a group of pesticides have been associated with many cancers. As of 2014, over 250 million pounds of glyphosate, an active ingredient in the popularized herbicide product, Roundup® (RU), has been used across the United States. Although pesticides are known to come with benefits for agriculture, such as pest control and increased food production, in contrast it has been proven to be a human health concern. While studies have shown multiple toxic effects in increased correlation between diseases with RU and glyphosate exposure, the full extent of its hazard is still being researched. According to the US Environmental Protection Agency (US EPA), the legal limit of glyphosate is 140 mg/kg. This does not take into consideration the risk of RU at low doses. Thus, this study aims to examine a broad range of dose responses, below what is considered the safe limit or threshold of RU, using the deletion (DEL) assay. The DEL assay model is a eukaryotic (yeast strain RS112) in vitro model that examines a double stranded break in the yeast when exposed to a toxic substance. This model is inexpensive, easy to apply, and a single assay that looks at the Shikimate pathway to study genotoxic and cytotoxic effects. By exposing RS112 to RU concentrations between 100mg/mL to 0.001pg/ml we have observed a nonmonotonic
Pesticide use in Thailand has increased annually and many of these pesticides are potentially immuno- or neurotoxic in humans. For this project, hospital staff and health volunteers were assisted with recruitment: contacting farms, collecting samples, and conducting medical examinations. All study participants—conventional farmworkers (n=70) and age and sex-matched comparison workers (n=70)—were enrolled in this study. Conventional farmworkers were selected based on the previous study, and comparison workers were selected from the general population. The study was approved by the Institutional Review Board at both the University of Michigan and the University of New Orleans. Serum samples were collected from all participants at baseline and at the end of the 10-day study period. Blood and urine samples were collected from all participants at baseline, at the end of the study period, and at the follow-up visit. The samples were analyzed for a wide range of pesticides using high-performance liquid chromatography and tandem mass spectrometry. The results showed that conventional farmworkers had significantly higher levels of exposure to pesticides than comparison workers. This study highlights the importance of pesticide exposure studies in developing countries and the need for effective public health interventions to reduce pesticide exposure. 

**References**


**Pesticide Use in Thailand**

Myclobutanil, a conazole class fungicide is heavily used to control fungi and fungal diseases associated with perennial and annual crops, turf, fruit trees, and vines by inhibiting ergosterol synthesis. Conozoles are known to have hepatotoxic effects in mice through induction of hepatic cell proliferation and altered cholesterol levels. Myclobutanil is non-tumorigenic in mice and is considered to have low acute toxicity in humans. However, the mechanisms of the hepatotoxic effects of the fungicide are poorly understood. Most recently, myclobutanil has been associated with increased lipid deposition in hepatocytes and cell death in an in vitro model, suggesting the environmental contaminant can impact hepatocellular diseases. In this study, myclobutanil exposure was assessed as a potential contributor to non-alcoholic fatty disease (NAFLD), specifically subacute changes in genes associated with NAFLD in the liver and white adipose tissue were assessed. C57BL/6J mice at 18 weeks of age were dosed with vehicle, 20 ppm, 100 ppm, or 500 ppm of myclobutanil (CAS Number : 88671-89-0) via oral gavage for 10-days and sacrificed 3 hours following the last dose. In agreement with previous studies, there was a trend for a dose-dependent increase in liver to body weight percentage that achieved significance compared to vehicle at 70.2 mg/kg/day (53%, p=0.005) and a trend for increased alanine aminotransferase (ALT) serum levels with increasing dose, with ALT levels of 63±28 IU/L at 500 ppm compared to vehicle levels of 33.2±8 IU/L. Key genes of the bile acid synthesis pathway, Cyp7a1 and Cyp8b1, were up-regulated with myclobutanil exposure. Cyp8b1 was significantly increased for vehicle at all doses, and Cyp7a1 was significantly increased 14.5 and 17-fold at 100 and 500 ppm doses, respectively. In white adipose tissue, a trend for increased mRNA levels of Pparγ2, Adiponectin, and aP2 at the highest exposure was noted. The changes in mRNA levels following a subacute oral exposure to myclobutanil suggest that the nuclear receptor FXR or the disruption of bile acid homeostasis may contribute to the hepatotoxicity in mice. Further, myclobutanil modulation of metabolic pathways within adipose tissue may favor adipogenesis contributing to altered lipid regulation. Therefore, the characterization of whether myclobutanil contributes to the enhancement of NAFLD disease progression is paramount.

**References**


**Cigarette Smoke Exposure in a Three-Dimensional Model of Human Bronchial Epithelial Cells**


Cigarette smoke (CS) is a complex mixture of chemicals and interacts with various physiological processes. We previously reported that nuclear factor erythroid 2-related factor 2 (NRF2) was the most sensitive transcription factor to aqueous cigarette smoke extract (AqCSE) exposure in monolayer cultured human bronchial epithelial cell lines. Recently, in vitro three-dimensional (3D) culture models have been used to supplement pharmacological and toxicological assessments. Bronchial epithelium models in particular are useful for the evaluation of substances that directly contact the respiratory tract, such as CS. In the present study, we used 3D-cultured human bronchial epithelial cells (HBEc3) to assess activation of transcription factors and relevant gene expression in response to AqCSE, primarily focusing on NRF2 and nuclear factor-kappa B (NF-kB) pathways. The 3D-cultured HBEc3 exposed to AqCSE showed expression of NRF2 and its nuclear translocation in addition to upregulation of genes related to oxidative stress. Our results suggest that the NRF2 pathway was the dominant pathway when 3D-cultured HBEc3 were exposed to AqCSE at a low dose, supporting our previous findings that NRF2 was the most sensitive transcription factor in response to AqCSE. Expression and nu...
clear translocation of NF-κB were not increased, although pro-inflammatory genes were upregulated. However, another inflammation-related transcription factor, activation protein 1 (AP-1), was induced by AqCSE. Gene classification analysis suggested that induction of the inflammatory response by AqCSE was dependent on NQO1 and AP-1 rather than NF-κB.

1928 Developing a Versatile Exposure System for the Analysis of the Effects of Electronic Cigarettes


Use of electronic cigarettes (e-cigs), particularly among youth, has proliferated in recent years. However, there remains little consensus on e-cigs’ health effects. With the plethora of devices, e-liquids, and flavorings available on the US market, there is a need for a controlled yet flexible in vitro exposure system which can accommodate the versatility of devices. This standardized exposure system will allow for more meaningful comparison between e-cig devices, e-liquid components, and devices operated at varying settings. The goal of this study was to develop and optimize a versatile in vitro e-cig exposure system and perform initial experiments to determine biological effects. Our exposure chamber is composed of a 3 L Plexiglas cube with an inlet for e-cig operation, an outlet attached to a vacuum line, and a fan for aerosol distribution. A series of mock exposures using a third-generation e-cig device at a rate of 2.5 LPM (Fig. 1) was performed to determine chamber parameters which yield even aerosol deposition while minimizing gas loss and disruption of aerosol nucleation. Average aerosol deposition across the 12 well plate was found to be 1.634 mg/cm² for propylene glycol (PG), 1.600 mg/cm² for glycerin (GLY), and 1.707 mg/cm² for a 55:45 PG/GLY mixture with no significant difference in deposition between the wells of the plate. Initial application of the exposure chamber included exposure of human bronchial epithelial cells to vaporized PG and GLY at ratios of 100% PG, 55:45 PG/GLY, and 100% GLY. PG and GLY are universal components of e-liquids, with 55:45 being a commonly used ratio among e-cig users. IL-6 and IL-8 transcript levels were analyzed in the exposed cells at varying time points. Our results indicate that exposure to PG aerosols increased IL-6 and IL-8 transcript levels at 2 hours post-exposure, suggesting an inflammatory response. Liquid Chromatography interfaced to Electrospray Ionization Mass Spectrometry is used to analyze the aerosol composition. The results of this study describe a novel and versatile in vitro e-cig exposure system that will allow for a controlled, replicable exposure to a variety of different e-cig aerosols, thus producing meaningful in vitro assessment of e-cig toxicity.

1929 Analyzing the Cellular Stress Response in Airway Epithelial Cells to Vaporized Propylene Glycol and Glycerol


In the United States the use of electronic cigarettes (e-cigs) has surged since its introduction to the market in 2007. Despite previous claims that e-cig use is ‘safer’ than traditional cigarette use, there is insufficient data to determine if e-cig generated aerosol itself is safe. To further compound the problem of determining the safety of e-cigs is the sheer number of variations in e-cig use: e-cig device type, wattage or temperature settings, flavorings and nicotine concentrations. However, one standard component of e-cigs, is its use of the humectants propylene glycol (PG) and glycerol (GLY). All e-cig devices heat the humectants to the point of vaporization, which can cause the formation of aldehydes and free radicals. The goal of this study is to determine the health effects of vaporized PG and GLY on airway epithelial cells. We exposed airway epithelial cells (16HBE cells) at air liquid interface to the vaporized humectants: 100% PG, 100% GLY, and 100% PG increased the expression of NAD(P)H Quinone Dehydrogenase 1 (NQO1) and the corresponding protein. These responses were not observed in 16HBE cells exposed to the aerosol generated at the 45W setting of the e-cig device. Additionally, we repeated the 80W exposure with primary airway epithelial cells from non-smokers and smokers. The 100% GLY exposure caused an upregulation of HMOX-1 in the cells from non-smokers but not smokers. Furthermore the PG/GLY exposure caused an upregulation of glutamate-cysteine ligase catalytic (GCLC), which is the rate limiting step of glutathione synthesis, in non-smokers but not smokers. These data suggest that aerosols generated from e-cig devices at high temperatures, regardless of flavoring content, are likely to cause cellular stress responses. Additionally non-smokers are likely to experience more cellular stress from e-cig use than smokers.

1930 Effects of E-cigarette Flavoring Chemicals on Human Macrophages and Bronchial Epithelial Cells

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E-cigarettes are a relatively new and popular alternative to tobacco cigarettes used by many young adults and teens. Due to their novelty, the respiratory health effects of e-cigarette flavoring components are not well understood. There are thousands of e-cigarette flavoring chemicals available on the market, many of which have the potential for toxicity. The majority of e-cigarette flavoring chemicals (ECFCs) have not been tested for inhalation safety. In this study, we used pulmonary-associated cell lines to assess the in vitro effects of thirty ECFCs to determine their potential cytotoxicity at various concentrations. The ECFC vehicles, propylene glycol (PG) and vegetable glycerin (VG), were tested individually and as mixtures that mirrored common ratios found in e-liquids (50:50 and 30:70 PG/VG, respectively). Cultured human monocytes (THP-1) were differentiated into a macrophage phenotype with vitamin D3 before treatment. Cultured human bronchial epithelial cells (BEAS-2B) and human monocyte-derived macrophages (HMMs) were treated with 10, 100, and 1000 μM of ECFC and analyzed for cytotoxicity and inflammatory markers, including changes in viability, cell membrane damage, reactive oxygen species (ROS) production, and inflammatory cytokine release. The ECFCs that caused the most cell death in both cell types (eugenol, linalool, and nonanol) were primarily classified as hydroxyls. A number of aldehydes (cinnamaldehyde, decanal, and trans-2-hexen-1-al) also caused significant cell death. Cell membrane damage, as measured by lactate dehydrogenase release, was elevated in both cell lines after treatment with eugenol, linalool, and nonanol. Decanal also caused membrane damage to BEAS-2B cells, while vanillen was damaging to THP-1 cells. Vanillien elicited high amounts of ROS from both cell lines, with the BEAS-2B also producing ROS after exposure to diketones (2,3-pentanedione, 2,3-heptanedione, and 2,3-hexanediol). These findings provide insight into the potential tissue damage that e-cigarette users are at risk for and provide a basis for future experiments with ECFC exposures.

1931 Systems Toxicology Assessment of a Representative E-liquid Formulation Using Human Primary Bronchial Epithelial Cells


Cigarette smoke (CS) is a risk factor for respiratory and systemic diseases, smoking cessation remains the most effective approach for risk reduction. Innovative products with the potential to reduce the risk of smoking related diseases are being developed with the aim to provide a better alternative to smokers who would otherwise continue to smoke. Literature suggests that e-vapor products likely have a significantly lower risk profile than cigarettes. E-vapor products that are novel in their notified high amounts of ROS from both cell lines, with the BEAS-2B also producing ROS after exposure to diketones (2,3-pentanedione, 2,3-heptanediol, and 2,3-hexanediol). These findings provide insight into the potential tissue damage that e-cigarette users are at risk for and provide a basis for future experiments with ECFC exposures.
and alpha-pinene (0.049%) were found to exhibit the highest cytotoxicity. A decrease in cytotoxicity was observed only when D-L-citronellol was removed, suggesting that D-L-citronellol was accountable for most of the mixture's overall cytotoxicity.

1932 Differential Regulation of Ion Channel Function from Exposures to Cigarette Smoke and ENDS Preparations

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Cigarette smoking is known to disrupt the normal mucociliary function of the lungs, whereas the effect of Electronic Nicotine Delivery Systems (ENDS) is incompletely understood. This study aimed to compare the effects of acute exposure of primary normal human bronchial epithelial (NHBE) 3D cultures at air-liquid interface to combustible cigarette and ENDS preparations on mucociliary function including ion channel function, ciliary beat frequency (CBF) and airway surface liquid (ASL) height. Differentiated NHBE cultures were exposed to whole smoke conditioned media (WS-CM) or total particulate matter (TPM) prepared from 3R4F reference cigarettes, whole aerosol conditioned media (ACM) or e-TPM generated from a marketed ENDS product, or nicotine alone. We found that a dose of 7μg/ml equi-nicotine units of cigarette TPM and WS-CM significantly decreased Cystic Fibrosis Transmembrane conductance Regulator (CFTR) and the epithelial sodium channel (ENaC) function which regulate fluid homeostasis in the lung. Conversely, higher (5μg/ml) equi-nicotine units of ENDS preparations or nicotine alone had no effect on CFTR and ENaC function. Despite a significant decrease in ion channel function, cigarette smoke preparations did not alter CBF and ASL. Similarly, ENDS preparations and nicotine alone had no effect on ASL and CBF. This study demonstrates that acute exposures of cigarette smoke preparations exert a notable inhibitory effect on the CFTR and ENaC function compared to ENDS preparations. In summary, the functional assays described herein are useful for tobacco product evaluations.

1933 In Vitro Toxicity Induced by JUUL and E-cigarette Aerosol Extracts in Human Bronchial Epithelial Cells

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While electronic-cigarettes (e-cigs) are open system devices with user-adjustable operational conditions, JUUL devices are closed system with no modifiable settings. These devices of different design are the most commonly used form of tobacco products by youth in the US; however, scientific evidence related to the effects of e-cig or JUUL aerosols on lung toxicity is limited. The aim of this study was to evaluate the in vitro toxicity of two commonly used JUUL and e-cig flavors: cool mint, a simple flavor mixture, and crème brûlée, a more complex flavor mixture, on human bronchial epithelial (BEAS-2B) cells. JUUL and e-cig aerosol extracts displayed a concentration-dependent decrease in lactate dehydrogenase (LDH) activity (≥25%), a marker of cytotoxicity, for cool mint and crème brûlée JUUL and e-cig aerosol extracts vs. control. Moreover, gene expression (qRT-PCR) were assessed. Cool mint and crème brûlée JUUL flavors composed of a mixture of catechol, a polyphenol, a natural pharmaceutical ingredient, and IL-10, an anti-inflammatory cytokine, were increased ≥4 fold by JUUL and e-cig aerosols compared to control. While the adaptation of existing cigarette smoker-based testing puffing regimes to alternative nicotine delivery devices are an attempt at controlling variability, these fail to address variables unique to the devices themselves such as wattage or flow rate. Furthermore, it is vital to match puff regime to user behavior for realistic exposure assessment. The study investigated variability of aerosol mass generation in two dissimilar devices: the JUUL, a popular electronic cigarette, and a Vaporeoso custom tank aimed at vaping enthusiasts, with the ultimate goal of examining toxicity in 2D and 3D cell culture systems. Both e-cigarette devices were coupled with an electronic cigarette aerosol generator (eAerols) for the generation of an aerosol which was captured on in-line 44nm Cambridge filter pads and weighed. The JUUL device was driven according to the CORESTA puffing regime. JUUL aerosol generation was found to vary considerably with increasing puff count on a single fluid filled pod (1.69mg at puff Number 1 to 3.18mg by puff Number 160), between puffs on the same pod (5D from 0.14 to 1.87mg), and between pods (range 2.17mg to 4.10mg). Such variability only becomes apparent when flow rate and puff timing are strictly controlled. The Vaporeoso tank was subjected to both the CORESTA regime (3s coil activation, 55ml puff volume, 30s interval) and a more realistic profile based upon user observation (4s coil activation, 500ml puff volume, 30s interval) while varying total power output from 60 to 110W. The CORESTA regime revealed an initial increase of aerosol generation with increase in power leveling off between 80 and 110W (17mg initial, leveling at 23mg). The more realistic puff profile demonstrated much higher mass production and a linear increase with no plateau effect (47mg to 92mg). Consequently, applying the CORESTA method to this device appears to underestimate user exposure. Thus, toxicologists must validate e-cigarette aerosol generation methodologies for reproducible toxicity testing.

1934 The Importance of Controlling Exposure Generation Parameters for In Vitro Electronic Cigarette Toxicity Testing

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Electronic cigarette use increases annually with now soaring rates seen among underage demographics. The current number of studies are insufficient to address health concerns arising from widespread use. Inter-study variability creates doubt about the validity or inter-comparability of study results. Meaningful design requires reliable and replicable control of all parameters influencing exposure generation to study the effects of e-cigarette aerosol on cell culture models. While the adaptation of existing cigarette smoker-based testing puffing regimes to alternative nicotine delivery devices are an attempt at controlling variability, these fail to address variables unique to the devices themselves such as wattage or flow rate. Furthermore, it is vital to match puff regime to user behavior for realistic exposure assessment. The study investigated variability of aerosol mass generation in two dissimilar devices: the JUUL, a popular electronic cigarette, and a Vaporeoso custom tank aimed at vaping enthusiasts, with the ultimate goal of examining toxicity in 2D and 3D cell culture systems. Both e-cigarette devices were coupled with an electronic cigarette aerosol generator (eAerols) for the generation of an aerosol which was captured on in-line 44nm Cambridge filter pads and weighed. The JUUL device was driven according to the CORESTA puffing regime. JUUL aerosol generation was found to vary considerably with increasing puff count on a single fluid filled pod (1.69mg at puff Number 1 to 3.18mg by puff Number 160), between puffs on the same pod (5D from 0.14 to 1.87mg), and between pods (range 2.17mg to 4.10mg). Such variability only becomes apparent when flow rate and puff timing are strictly controlled. The Vaporeoso tank was subjected to both the CORESTA regime (3s coil activation, 55ml puff volume, 30s interval) and a more realistic profile based upon user observation (4s coil activation, 500ml puff volume, 30s interval) while varying total power output from 60 to 110W. The CORESTA regime revealed an initial increase of aerosol generation with increase in power leveling off between 80 and 110W (17mg initial, leveling at 23mg). The more realistic puff profile demonstrated much higher mass production and a linear increase with no plateau effect (47mg to 92mg). Consequently, applying the CORESTA method to this device appears to underestimate user exposure. Thus, toxicologists must validate e-cigarette aerosol generation methodologies for reproducible toxicity testing.

1935 A 7-Month Inhalation Study in C57Bl/6 Mice to Investigate Potential Toxicity of E-vapor Aerosols Compared to Cigarette Smoke Using Cessation and Switching Study Design

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Chronic exposure to cigarette smoke is a risk factor for the development of various diseases. Smoking cessation remains the most effective approach to minimize smoking related diseases, however only a small percentage of smokers succeed in quitting. It is generally regarded that switching from combustible cigarette to e-cigarettes (e-vapors), can offer a potential alternative for tobacco-related harm reduction. Though various e-vapor products are available, studies evaluating long-term toxicity and biological implications of e-vapors alone and in the context of switching from cigarettes to e-vapors are not available. We designed a 7-month nose only inhalation (4 hours/day, 5 days/week, for 7 months) study to evaluate chronic toxicity of MarkTen® e-vapor aerosols and compared it to responses from exposure to the 3R4F reference cigarette (CS). Additional groups of mice were added to explore the impact of switching or cessation after first 3 months of exposure to CS. Over the 7 months of exposure, there were no notable in-life observations from the e-vapor groups. Their body weights were comparable to the sham control, whereas the CS group showed consistently lower body weight. In contrast to e-vapor, the 3R4F group showed transient clinical signs of distress post-exposure, and, in part because of reduced respiratory volume during exposure, they had lower plasma nicotine and cotinine levels compared to the e-vapor group. In addition, the CS exposure induced significant changes in organ weights. Following 7 months of exposure, e-vapor resulted in no or minimal increase in inflammatory cellular responses in BALF, while the CS group showed consistently elevated responses (activated macrophages, CD4+ T cells, neutrophils and eosinophils). Also, the extent of changes observed in switching group was overall similar to changes observed in the cessation group. Altogether, the pathophysiological changes induced by e-vapor exposures were significantly lower than changes induced by CS expo-
1936 Toxic Effects of Waterpipe Tobacco Smoking: Revealing the Mechanism Using Bioinformatics Approach

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Waterpipe tobacco smoking (WTS), in Middle East commonly called shisha or narghile, is becoming more popular in Western societies, especially among young people as an alternative form of tobacco use other than traditional cigarettes. Although waterpipe tobacco use is associated with greater carbon monoxide, similar nicotine, dramatically more smoke exposure and some of the same toxins as cigarette smoking, the health risk associated with WTS is highly underestimated. The aim of this study was to investigate toxicities induced by light-use WTS. We analysed gene expression data (Walters M et al, 2017) from small airway epithelium of waterpipe tobacco smokers and WTS impact on pathological changes and affected pathways. From the whole genome analysis, 282 signature genes (p<0.05, FC=1.5) were detected as significantly dysregulated after exposure to WTS. We created a computational model of biological pathways describing cellular processes in human respiratory tissues, by manually annotating and processing molecular information from the published data (PubMed articles and UDA reports) and making the information computable. WTS exposure induced genes involved in immune responses, G-protein coupled receptor signalling, oxidative stress and mRNA regulation of translation. Furthermore, we annotated data about WTS toxic components (such as the ones from Hoffmann’s list, Hoffmann D et al, 1998) and generated a comprehensive database of known side effects and protein targets of these toxicants. By applying bioinformatics analysis tools to gene expression data and combining with toxicants data, we have identified several pathologies that affect respiratory and circulatory system. For each of these pathologies we generated the hypothesis of mechanism of action that is supported by current knowledge. These data imply that even light-use waterpipe tobacco smoking is as much harmful as traditional cigarette smoking, and may lead to damaging respiratory system.

1937 Prenatal Waterpipe Tobacco Smoke Exposure Alters Lung Immune Responses to House Dust Mite Allergen in Adult Offspring Mice

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The rate of waterpipe (hookah) use is as high as 40% among young adults of reproductive age. There is limited amount of research evaluating potential adverse health effects resulting from maternal hookah use during pregnancy. This study was designed to test the hypothesis that in utero exposures to waterpipe tobacco smoke (WTS) aggravate adult lung immune responses to house dust mite (HDM) allergen. Pregnant BALB/c mice were exposed to either air or WTS with average CO levels of 633 ppm for 2 hours per day during gestational days 6 to 19. Starting at 14 and 15 weeks of age, male and female offspring, respectively, were given an intranasal instillation of saline or HDM (males = 50 μg, females = 25 μg) once a week for 3 weeks. At 17 and 18 weeks of age, lung function (plethysmography), bronchoalveolar lavage fluid (BALF), and lung tissue were examined. In utero exposure status did not alter lung function or structure. Of 84 allergy- and inflammation-related genes examined in the lungs via qPCR array, in utero WTS exposure alone resulted in a significant down-regulation of 24 genes in adult males and 11 in adult females. The 9 genes common to both males and females (Adam33, Alox5, Bcl6, C1r/c2, Il-3ra, Il-4ra, Stat6, Tbx1, and Trnfsf4) are cytokine receptors and transcription factors involved in Th1/Th2 activation and Jak1/Jak3 cytokine signaling pathways, indicating early downstream immunosuppression. In males, histopathological assessment of both HDM challenged groups revealed phenotypic changes associated with allergic asthma. Compared to its in utero air + HDM controls, in utero WTS + HDM males had significantly higher levels of BALF neutrophils and increased gene expression of Il-13 and Il-21 (> 5-fold), and Cc4 and Cc11 (> 3-fold), suggesting an exacerbation of HDM-induced neutrophilic inflammation due to in utero WTS exposure. In the lungs of in utero WTS + HDM females, Cc256, Cc2f, Il-17a, and Il-25 were up-regulated (> 3-fold) compared to the in utero air + HDM females, yet only the latter displayed phenotypic asthmatic responses. No significant cellular or tissue changes were observed in the in utero WTS + HDM females, suggesting a sex-specific response of males to WTS plus HDM allergen. Overall, our data suggest that prenatal exposure to WTS modifies the adult immune response to a common respiratory allergen and that in utero WTS exposures alone may have immunosuppressive effects in the lungs that persist into adulthood.

1938 Exposure of Cells of the Airways to Cigarette Smoke Extract or Acrolein Disrupts the Molecular Regulation of Mitochondrial Metabolism

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Chronic Obstructive Pulmonary Disease (COPD) is a devastating, highly prevalent and incurable lung disease mainly caused by smoking. During pyrolysis and combustion of tobacco, various types of aldehydes are generated of which, due to its unsaturated structure, acrolein is considered the most reactive. From in vitro studies it is known that acrolein can induce cellular processes considered to be central to COPD pathogenesis including inflammation, oxidative stress, cell death and tissue remodelling. Interestingly, mitochondria, traditionally viewed as the cell’s powerhouse, are now known to play an important regulatory role in all of these processes. Moreover, mitochondrial morphology was found to be abnormal in airway epithelial cells of COPD patients. Based on this, we hypothesized that cigarette smoke-induced mitochondrial dysfunction, driven by aldehydes, is the central mechanism underlying COPD airway pathogenesis. This was addressed by investigating the expression of genes involved in the regulation of mitochondrial metabolism, assessment of activity of mitochondrial metabolic enzymes and abundance of mtDNA copy number in primary human bronchial epithelial cells (PBECs), provided by PLUC facility MUMC+, exposed to cigarette smoke extract (CSE: 0-1-2-4%) as well as in lung tissue of rats exposed acutely (1-2 days) or sub-acutely (4 weeks) to acrolein (0-4 ppm). CSE dose- and time-dependently affected gene expression of key constituents and regulators of mitochondrial metabolism in PBECs. Moreover, both acute and sub-acute in vivo exposure of rat lungs to acrolein dose- and time-dependently decreased the activity of Hydroxacyl-Coenzyme A dehydrogenase (rate-limiting enzyme in the mitochondrial fatty acid β-oxidation), upregulated mtDNA copy number and affected mRNA levels of genes involved in the molecular regulation of mitochondrial metabolism. These results show that CSE as well as acrolein disturb the molecular regulation of mitochondrial metabolism in cells of the airways. Disclaimer: This work does not reflect US EPA policy.

1939 Acute Exposure to Thirdhand Smoke Produces Rapid Changes in the Human Nasal Epithelial Transcriptome

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Thirdhand smoke (THS) is the residue that builds up on indoor surfaces after smoking has occurred. These chemicals remain for months or years and non-smokers can be exposed unknowingly. Prior studies on the toxicity of THS have been done with cultured cells and animal models. The purpose of this study was to evaluate the effects of acute THS exposure on the transcriptome of the human nasal epithelium. Non-smoker females were exposed for a total of ~3 hours in controlled laboratory conditions, first to clean air and later to THS. Nasal scrapes taken before and after exposure were analyzed for differential gene expression using RNA-seq. Clean air exposure only affected the expression of two genes. In contrast, in THS exposed nasal epithelium, 389 genes were differentially expressed, some of which are associated with increased mitochondrial activity, oxidative stress, DNA repair activity, cell survival, and inhibition of cell death. Activation of some of these processes may be due to residual nicotine in THS. Several of these responses are similar to those demonstrated previously using in vitro cultured cells, such as stress-induced mitochondrial hyperfusion. In conclusion, our translational study is the first to show that a 3 hour exposure to THS affects gene expression in humans. Our data are consistent with the idea that short exposure induces cell stress leading to the activation of various survival pathways and that nicotine may be involved in promoting survival. These data will be valuable in formulating regulations for the remediation of THS contaminated environments.
**1940** Cigarette Smoke Induced Pathophysiological Changes in the Extracellular Matrix but not Inflammation as Early Events in Fresh Human Lung Tissue

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Cigarette smoke (Cs) inhalation is a main reason to develop chronic obstructive pulmonary disease (COPD), characterised by degradation of alveoli, mucus hypersecretion, emphysema development and inflammation [1]. The pathophysiology of COPD is not well understood so the mechanisms that underlie various components of COPD need to be modelled in vitro, specifically using Cs. We assessed the pathophysiological effects of Cs and Csc on fresh human lung tissue. Human Precision Cut Lung Slices (PCLS) were exposed to Cs or Csc to determine whether their toxicological effect on the lungs. Mitochondria maintain the cellular homeostasis by balancing metabolic activities. We determined the molecular events in the process of Cs-induced mitochondrial dysfunctions and mitochondrial function and dynamics leads to the disruption of cellular function. This study emphasizes the crucial role of mitochondrial proteins and bioenergetics in Cs-induced lung epithelial cellular stress and senescence. Supported by the NIH grants HL137738-01, HL135613, HL085613, ES028006, and EB023595.

**1941** Mitochondrial Stress and Dysfunction by Tobacco Smoke, Leading to Cellular Senescence in Lung Epithelial Cells


Tobacco smoking remains one of the major causes of COPD, which is debilitating and the current treatment is palliative. Cigarette smoke (CS) contains several chemical and physical toxins that are responsible for their toxicological effect on the lungs. Mitochondria maintain the cellular homeostasis by balancing the cellular respiration and energy demand. Any pathological insults to the mitochondrial function and dynamics leads to the disruption of cellular functions. We hypothesized that Cs induces mitochondrial dysfunction in several lung epithelial cells leading to cellular abnormalities. We determined the molecular events in the process of Cs-induced mitochondrial dysfunctions and mitostress by the Seahorse technique. Methods: Human pulmonary epithelial cells like SAEC, NHBEAC, BEAS2B, and mouse MLE15 were exposed to Cs or Csc (CSE) at varying lengths of time (from 24hrs to 15days) and concentrations (0.25% to 2%) and observed various parameters for several endpoints. A minimum of three samples from each group were analyzed for all the estimations. Results: CSE treatment significantly decreased several mitochondrial parameters like non-mitochondrial oxygen consumption (P<0.01), basal respiration (P<0.05), ATP production (P<0.05), and spare respiratory capacity (P<0.05) in these cell lines. OXPHOS blot analysis showed decrease in several of the mitochondrial protein complexes exposed to CSE. Experimental analysis using mitochondrial specific dyes showed decrease in mitochondrial mass, membrane potential, along with the increase in mitochondrial ROS production leading to cellular senescence at day 15. Western blot analysis showed the involvement of important mitochondrial proteins, such as Rho TPase (Miro1), mitofusin2, and parkin in mitochondrial dysfunction caused by CSE (24hrs and 15days). Further studies employing RHO zero cells determined the precise role of mitochondrial disruptions/transfers in these cells. Seahorse data for mitostress and phenotype characterization indicated that cells are under stress at different doses and duration of Cs exposure (P<0.05).

**1942** Vaping during Pregnancy Impairs Cerebrovascular Function in Offspring

E. N. Burrage, E. A. Aboaziza, M. C. Parsley, K. Marshall, J. Moore, A. Johnson, J. O’Reilly, L. Hare, S. Dangott, A. Tice, P. D. Chantler, and L. Oliert. West Virginia University School of Medicine, Morgantown, WV.

Electronic cigarettes (Ecigs) have gained popularity amongst the young, and even is being promoted as a safe or ‘safer’ option than smoking tobacco cigarettes during pregnancy. However, it is unknown if Ecigs are a safer option during pregnancy. We examined the effects of maternal Ecig exposure (Joyetech eGrip OLED using 5-sec puffs @17.5 W) on cerebrovascular function in offspring from rat dams that had been exposed ambient air (Sham, n=6), Ecig with 18 mg/ml nicotine (Ecig+18, n=5) and without nicotine (Ecig+0, n=5). Exposure consisted of 30 puffs over 1-hour each day, 5 days/week, and resulted in an average daily TPM of ~120 mg/m². Maternal exposure was started on gestational day 2 and continued until pups were wean. Pups were never directly exposed. The middle cerebral arteries (MCA) were obtained from 1-month old pups, isolated and positioned in a pressurized in myobath, and exposed to increasing concentrations of acetylcholine (Ach; 10⁻⁴ M to 10⁻² M), serotonin (5-HT; 10⁻⁴ M to 10⁻² M), and sodium nitroprusside (SNP; 10⁻⁴ M to 10⁻³ M) in the presence or absence of Tempol (a superoxide dismutase mimetic). The MCA dilation to Ach was impaired in Ecig+0 and Ecig+18 vs sham pups (14±2%, 10±1%, 30±2%, p<0.05, respectively). Tempol did not affect MCA dilation to Ach in either sham or Ecig+0 pups, but restored MCA dilatory response to Sham levels in Ecig+18 pups, suggesting that vascular dysfunction associated with exposure to Ecig with nicotine involves the superoxide pathway. The MCA constriction to 5-HT was similar between all groups. Minor changes were evident with endothelium-independent dilation to SNP between groups. Examination of posterior cerebral arteries incubated in diaminofluorescein-(DAF)-FM showed a lower nitric oxide bioavailability in the Ecig+0 and Ecig+18 vs pups exposed to nicotine (3.0±2.3, 1.3±0.3 vs 17.3±6.1 au, respectively, ANOVA p<0.05). These data suggest offspring born to mothers who vape during pregnancy (with or without nicotine) have impaired cerebrovascular function suggesting it may not be safe. Our data also suggest the pathway(s) involved may be different with vs without nicotine. Support: WVU Cancer Institute Philip R Dino Innovative Research Grant (IMO); APS STRIDE Fellowship (JD); NIH/NIAMS S52GM101942-03 (DC).

**1943** In Utero Exposure to Electronic Cigarettes Results in Aortic Dysfunction

E. A. Aboaziza, E. N. Burrage, J. Moore, J. O’Reilly, M. C. Parsley, K. Marshall, A. Johnson, L. Hare, S. Dangott, A. Tice, P. D. Chantler, and I. M. Oliert. West Virginia University School of Medicine, Morgantown, WV.

Electronic cigarettes (Ecigs) are touted as effective smoking cessation tools and are popular among women of child-bearing age, despite recent evidence of cardiovascular harm. Pregnant women smokers alarmingly turn to Ecigs during pregnancy, although risks to their offspring (even when using nicotine-free e-liquid) are largely unknown. Rat dams were exposed to either nicotine-free Ecig vapor (Ecig+0), Joyetech eGrip OLED using 5-sec puffs @17.5 W or ambient air. Exposure consisted of 30 puffs over 1-hour each day, 5 days/week, and resulted in an average daily TPM of ~120 mg/m². Maternal exposure started on gestational day 2 and continued until pups were weaned (postnatal day 21). Pups were never directly exposed. 2mm segments of thoracic aorta were obtained from 3-month old pups (Ecig+0, n=8; Sham, n=8), dissected out, and mounted on a wire myograph system (DAM, AD Instruments) containing warmed aerated Krebs-Henseleit buffer solution. Vessels were pre-constricted with U46619 (10⁻⁴ M) and exposed to increasing concentrations of methacholine (Mch;10⁻⁵ M to 10⁻³ M) to assess endothelial-dependent relaxation. Pre-constricted vessels were challenged with sodium nitroprusside (SNP;10⁻⁴ M to 10⁻³ M) for endothelial-independent relaxation. Additionally, the rings were subjected to increasing doses of U46619 (U46;10⁻⁴ M to 10⁻³ M) for a constriction-dose response (reported as a % of maximum constriction to KCl). Significant impairment in thoracic dilation to Mch was observed in Ecig pups vs Sham pups (78.6±4.6% vs 86.6±6.0%, p<0.05). No difference in constriction to U46619 nor change in SNP endothelial-independent dilation were different between groups. Examination of aortic rings incubated in diaminofluorescein-(DAF)-FM showed a lower nitric oxide bioavailability in the Ecig+0 pups vs air exposed pups (3.0±2.3, 1.3±0.3 vs 17.3±6.1 au, respectively, ANOVA p<0.05). These data suggest offspring born to mothers who vape during pregnancy (with or without nicotine) have impaired cerebrovascular function suggesting it may not be safe. Our data also suggest the pathway(s) involved may be different with vs without nicotine. Support: WVU Cancer Institute Philip R Dino Innovative Research Grant (IMO); APS STRIDE Fellowship (JD); NIH/NIAMS S52GM101942-03 (DC).
In vitro studies have supported the toxicological evaluation of chemicals and complex mixtures including cigarette smoke and next generation tobacco and nicotine products (NGP) which include electronic-cigarettes and tobacco heating products (THP). The NGP environment is fast-paced requiring higher throughput and more advanced in vitro studies, to meet the growing requirement for product innovation and the duty of care assessments. These products are believed to be less risky compared to conventional cigarette smoking, but more work is required to understand this new and continually evolving category. In this study, total particulate matter (TPM) from two commercially available THPs (termed THP1.0 and THS) and a Kentucky reference (3R4F) cigarette were assessed using the in vitro micronucleus assay under various conditions and cell types (V79, TK6 and CHO-K1). V79 and TK6 cells were assessed under short +/- 59 and long +/- 59 conditions using standard manual scoring techniques. CHO-K1 cells were assessed under short +59 conditions and long -59 conditions using automated cell imagine high content screening approaches (Cellomics ArrayScan VTI). The response to reference cigarette smoke varied between cell types. V79 cells gave the most consistent response with all three treatment conditions producing a clear positive response. Human TK6 cells only produced a weak-positive response under one condition (3hr+59) and CHO-K1 cells produced a positive response under long -59 conditions, with automated scoring. However, all three cell lines equally demonstrated a negative response with THPs up to 500 µg/mL. In many cases toxicity of the test article indicated that treatment conditions could be pushed even further. In conclusion, the THPs assessed did not increase the micronucleus formation above control levels at TPM doses far exceeding that of cigarette smoke and up to 500 µg/mL. Cigarette smoke responses were observed in the 0-70 µg/mL range depending on cell type used. This study further supports the growing consensus that THPs are potentially less risky than conventional cigarettes and that innovative screening technologies like HCS can be employed for NGP product assessment. This will become especially important where increasing product innovations require higher throughput duty of care in vitro assessments.

In this study, traditional in vitro toxicological approaches such as in vitro mutagenicity, cytotoxicity and tumour-promoting activity assays were employed across total particulate matter (TPM) and whole aerosol text matrices, to assess cigarette smoke (Kentucky Reference 3R4F) against a prototype electronic cigarette (e-cigarette) that has a unique aerosolisation technology. The Ames bacterial mutation assay was employed using Salmonella typhimurium tester strains TA98, TA100, TA1535, TA1537 and TA102 +/- metabolic activation (59). The mouse lymphoma assay (MLA) was assessed +/- metabolic activation with short 3 h exposures and longer 24 h -59 exposures. The Bhas 42 cell transformation assay supplemented traditional approaches and was incorporated as an in vitro alternative for detecting tumour promoters and the neutral red uptake (NRU) cell viability assay provided an acute measure of cytotoxicity. The in vitro micronucleus assay was employed +/- 59 with short and long exposures. To complement this testing strategy, the Ames assay was also employed with S. typhimurium strains TA98, TA100, TA1535, TA97 and TA102 using a scaled down 35 mm whole aerosol methodology. This methodology utilised a unique set of undiluted exposure parameters. 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 60 µg/mL, 240 µg/plate, 60 µg/mL, 50 µg/mL and 30 µg/mL respectively. In contrast, THP TPM failed to elicit a response in each of the assays up to 500 µg/mL. In a complementary approach undiluted e-cigarette aerosol was assessed using 90%Bufs. Despite delivering a more concentrated aerosol stream under extreme exposure conditions, e-cigarette aerosol was deemed negative under all conditions and strains, confirming the results obtained in TPM assessments. These data demonstrate that the e-cigarette assessed was negative at doses equivalent and exceeding those of cigarette smoke where positive responses are observed in all assays assessed. This study further supports the growing consensus that e-cigarettes are significantly less toxic than cigarette smoke and that a novel technological development in this case did not adversely affect the genotoxicological outcome.
E-cigarettes (e-cigarettes) are often marketed as safer than tobacco products and are gaining popularity as an alternative to smoking traditional cigarettes. E-cigarettes heat and vaporize e-liquids, which typically contain humectants, nicotine, and flavorings. The flavorings found in e-liquids are generally recognized as safe (GRAS) for oral consumption by the United States Food and Drug Administration (US FDA) but have not been evaluated for inhalational toxicity. Our lab has previously shown that the flavoring chemical cinnamaldehyde, an aromatic aldehyde that is component of spicy and cinnamon-flavored e-liquids, can impair key functions in human neutrophils, macrophages, and natural killer cells. In this study, we isolated peripheral blood neutrophils from healthy human subjects and investigated the effects of two other aromatic aldehyde flavoring chemicals found in e-liquids - ethyl vanillin and benzaldehyde - and one non-aromatic aldehyde flavoring - isoamyl acetate. We challenged the isolated neutrophils with up to 5 mM of each flavoring compound and then assessed changes in phagocytosis, oxidative burst, and cytotoxicity.

Materials and Methods:

Neutrophils were isolated from whole blood samples using a density gradient centrifugation method. The isolated neutrophils were then challenged with 5 mM of each flavoring compound and the effects on phagocytosis with pHrodo Red-labeled Staphylococcus aureus particles, oxidative burst, and cytotoxicity were assessed.

Results:

1. Phagocytosis: The percentage of neutrophils that phagocytosed pHrodo Red-labeled S. aureus particles was significantly decreased in the presence of ethyl vanillin and benzaldehyde compared to control, while isoamyl acetate did not significantly affect phagocytosis.

2. Oxidative Burst: The ROS production measured by dihydroethidium (DHE) was significantly decreased in the presence of ethyl vanillin, benzaldehyde, and isoamyl acetate compared to control.

3. Cytotoxicity: The lactate dehydrogenase (LDH) release was significantly increased in the presence of ethyl vanillin, benzaldehyde, and isoamyl acetate compared to control.

Conclusion:

The study findings suggest that the aromatic aldehydes found in e-liquids can significantly impair phagocytosis and oxidative burst, and cytotoxicity of neutrophils. These findings highlight the potential respiratory health risks associated with e-cigarette use and underscore the need for further research to better understand the health impacts of e-cigarette flavorings.
1952 The Role of PI3K Pathway and MAPK Pathway in Cigarette Smoke-Induced Autophagy and Inflammation in BEAS-2B Cells

Smoking, as an individual consumption habit and cigarette smoke being environmental pollutant, involves a variety of related diseases. Exposure of cigarette smoke was reported to induce autophagy and inflammation in humans, experimental animals and different cell lines. However, the exact toxicological mechanisms of cigarette smoke in organisms have not been fully explored. In the present study, our goals were to explore the autophagy and inflammatory responses induced by cigarette smoke in human bronchial epithelial cells (BEAS-2B), and the role of the phosphatidylinositol 3-kinase (PI3K) pathway and the mitogen-activated protein kinase (MAPK) pathways in autophagy and inflammation. The alterations of autophagy-related proteins and inflammation-related proteins were detected by western blot assays, and the release of inflammatory cytokines was detected by enzyme-linked immunosorbent assays. The results showed that in BEAS-2B cells exposed to 10 µg/mL cigarette smoke particle matter (TPM), the activities of autophagy-related protein LC3B-II increased in time-dependent manner, and obtained its maximum at 24 h. The release level of IL-1β, IL-6 and IL-8 were increased with the exposure time. The alterations of autophagy classical pathway PI3K/Akt/mTOR were inhibited and inflammatory pathway MAPKs were activated in time-dependent manner. These results suggested that cigarette smoke-induced autophagy and inflammation, and the PI3K/Akt/mTOR pathway might be involved in the activation of autophagy induced by cigarette smoke, as well as the MAPK pathway might be involved in the inflammation induced by cigarette smoke. These results might help to understanding the adverse outcome pathway underlying cigarette smoke exposure and provide the information for the health risk assessment of tobacco products.

1954 Assessment of Anti-Cancer Potential of Nanogold Conjugated Toxin GNP-NN-32 from Naja Venom in Human Breast Cancer Cell Lines
S. Pandit, and S. Attarde. Savitribai Phule Pune University, Pune, India. Sponsor: P. Deshmukh

Cancer, despite the all-out efforts from developed countries still causes one in five deaths. Surgery, chemotherapy and radiotherapy provide inadequate protection and instead affect normal cells along with cancer cells. The present study aims at exploring the conjugation of protein toxin (NN-32) on GNP surface, characterization and comparative study of toxicity profile of NN-32 and GNP-NN-32. The present study was planned to evaluate in vitro toxicity of NN-32 and GNP-NN-32. The outline of study describes procurement of snake venom, purification of protein NN-32, the synthesis of gold nanoparticles (GNP), conjugation of NN-32 with GNP to synthesise GNP-NN-32 and characterization of GNP-NN-32 by various analytical methods. The study protocol was approved by the Institutional Bio-safety Committee (IBSC) and Institutional Animal Ethics Committee (IAEC) of Savitribai Phule Pune University, Pune, India. We have performed MTT assay, Neutral red uptake assay, Anti-proliferation assay and Detection of apoptosis by flow cytometric analysis on human breast cancer cell lines (MCF-7 & MDA-MB-231) after treatment with NN-32 and GNP-NN-32. IC50 values of NN-32 toxin and GNP-NN-32 toxin after 48 hrs treatment ranged between 2.5 and 6.7 µg/ml and 1.5 and 5.0 µg/ml for MCF-7 and MDA-MB-231 cell lines respectively, as compared to IC50 value 25µg/ml and 19 µg/ml respectively for the normal MCF-10A cells.

1955 Nicotine Induces Malignant Transformation through Oxidative Stress in Human Renal Epithelial Cells
Y. Chang, and K. Singh. Texas Tech University, Lubbock, TX.

Kidney cancer is one of the most common and lethal urologic malignancy. Nicotine is a component of cigarette smoke and mounting evidence implicates tobacco smoking in kidney cancer development. Whether nicotine itself can cause kidney cancer and the underlying molecular mechanisms is still not well understood. Hence, the objective of this study was to determine the chronic effects of nicotine exposure in malignant transformation of HK-2 kidney epithelial cells. In order to understand the molecular mechanism, the effect of nicotine exposure on the expression of genes for cellular reprogramming, redox status, and growth signaling pathways were also evaluated. Results revealed that chronic exposure to nicotine induced growth and malignant transformation in HK-2 cells. Interestingly, the nicotine-exposed cells had inherently (even after withdrawal of nicotine exposure) increased levels of intracellular ROS, acquired stem cell-like sphere formation, and epitheliomesenchymal-transition (EMT) changes. The analysis of gene expression further confirmed the results. Treatment with antioxidant NAC resulted in abrogation of EMT and stem cell-like sphere in HK-2 cells thereby suggesting the role of nicotine-induced ROS in these morphological changes. The results also suggest that nicotine-induced ROS, through regulation of AKT pathway, control the EMT and stemness during early stages of carcinogenesis. In summary, to our knowledge, this is the first report showing that chronic exposure to nicotine induces malignant transformation in human kidney epithelial cells through generating oxidative stress that can be potentially inhibited by antioxidant.

1956 DNA Transcription Highly Sensitizes the Del Assay to Ames Assay Negative Carcinogens
R. Schiestl. University of California Los Angeles, Los Angeles, CA.

Genetic instability is a hallmark of carcinogenesis. Furthermore, cells from patients carrying mutations conferring cancer prone phenotypes show a higher level of genetic instability, including DNA deletions. In fact, the original yeast-based DEL (deletion) Assay with 100 chemicals shows an accuracy of 92% to detect carcinogens as compared to 62% detected with the Ames Assay. DEL events in all three formats are inducible by a wide variety of carcinogens including carcinogens that are negative in many other short-term tests. Here we introduce the next generation DEL assay: a novel Saccaromyces cerevisiae strain, Del- Lac. During an exposure to a genotoxic event the sequence is excised and the two lacZ gene fragment showing an internal duplication of DEL-Lac. During an exposure to a genotoxic event the sequence is excised and the two lacZ gene fragment showing an internal duplication of DEL-Lac. During an exposure to a genotoxic event the sequence is excised and the two lacZ gene fragment showing an internal duplication of DEL-Lac. During an exposure to a genotoxic event the sequence is excised and the two lacZ gene fragment showing an internal duplication of DEL-Lac. During an exposure to a genotoxic event the sequence is excised and the two lacZ gene fragment showing an internal duplication of DEL-Lac. During an exposure to a genotoxic event the sequence is excised and the two lacZ gene fragment showing an internal duplication of DEL-Lac. During an exposure to a genotoxic event the sequence is excised and the two lacZ gene fragment showing an internal duplication of DEL-Lac. During an exposure to a genotoxic event the sequence is excised and the two lacZ gene fragment showing an internal duplication of DEL-Lac. During an exposure to a genotoxic event the sequence is excised and the two lacZ gene fragment showing an internal duplication of DEL-Lac. During an exposure to a genotoxic event the sequence is excised and the two lacZ gene fragment showing an internal duplication of DEL-Lac. During an exposure to a genotoxic event the sequence is excised and the two lacZ gene fragment showing an internal duplication of DEL-Lac. During an exposure to a genotoxic event the sequence is excised and the two lacZ gene fragment showing an internal duplication of DEL-Lac. During an exposure to a genotoxic event the sequence is excised and the two lacZ gene fragment showing an internal duplication of DEL-Lac. During an exposure to a genotoxic event the sequence is excised and the two lacZ gene fragment showing an internal duplication of DEL-Lac.
1957 Radiation Induces Microhomology Mediated Recombination In Trans


Ionizing radiation causes DNA double-strand breaks and genome rearrangements that can lead to cancer. Illegitimate recombination repairs a DNA double-strand break in the absence of extended sequence homology. Some illegitimate recombination events, also called microhomology-mediated recombination, occur between several basepairs of homology in the genome that generate about 50% of large deletions causing human genetic diseases. Ionizing radiation-induced genome rearrangements often show such microhomologies at their junctions, implying that radiation-induced double-strand breaks are preferentially repaired by such recombination events. However, events at undamaged sites have so far not been documented. Here, we report that both ionizing radiation and restriction enzymes induce microhomology-mediated integration of the DNA substrate in trans at sites that are not damaged. Irradiated yeast cells displayed 82% microhomology-mediated homologous integration, compared to only 27% in unirradiated cells. Restriction enzymes enhanced both integration events at genomic restriction sites and random non-restriction sites via microhomology-mediated recombination. Furthermore, exposure of yeast and mammalian cells to ionizing radiation before transformation of an end-joining substrate showed increased utilization of microhomology of end-joining events in a transfected plasmid that has not been exposed to radiation. These results suggest that genomic double-strand breaks caused by ionizing radiation or restriction enzymes induce a genome-wide microhomology-mediated illegitimate recombination pathway that facilitates integration in trans at non-targeted sites and might be involved in the generation of large deletions and rearrangements.

1958 The Extent to Which Sunscreen SPF Correlates with Ability to Protect against UV-Induced Mutations In Vitro


A commercial sunscreen’s sun protection factor (SPF) measures how much longer it protects skin against erythema induced by UVB light versus unprotected skin. Studies have shown DNA is a major chromophore for erythema, with erythema color intensity correlating with amount of DNA damage as measured by pyrimidine dimer formation (Young et al., 1998). However, the direct correlation between SPF level and protection against DNA damage has not previously been established. In this study, the relationship between SPF levels in commercial chemical sunscreen and protection against UVB-induced mutations was measured in vitro. Salmonella typhimurium TA 102 bacteria cells were exposed to UVB light with an SPF 15, 30, 50, or 100+ layer of sunscreen between the light source and petri dish. The number of revertant colonies (colonies) was counted after incubation, indicating the number of bacteria that mutated (Ames et al., 1973). Three trials at each SPF level were performed. For a UVB exposure of 45 seconds at 0.08 mw/cm², no significant differences were found between the mean number of colonies formed at each SPF level (SPF 15:295, SPF 30:358, SPF 50:395, SPF 100+376). The positive control, which was exposed to UVB light without sunscreen, had a statistically significant higher mean of 1671 colonies. A mean of 157 colonies formed in the negative control, which was not exposed to UVB light. The experiment was repeated with a longer exposure of 60 seconds at the same UVB intensity. Again, the mean number of colonies was not statistically different among SPF levels (SPF 15:530, SPF 30:581, SPF 50:539, SPF 100+469; pos. control: 2257, neg. control: 173). However, the means of the lower SPF levels (15:530 and 30:581) were higher than the means of the higher SPF levels (50:539 and 100+469), suggesting that high-SPF products may be more protective for longer exposures. This is expected based on the definition of SPF. How 45 to 60 seconds of UV exposure in a bacterium translate into hours of UV exposure hours in human is unknown. However, the results warrant further research into the exposure duration at which higher-SPF products become more protective against mutagenesis than lower-SPF products. Further research could guide the selection of the SPF sunscreen level necessary for protection against mutagenesis for the duration of time spent outside. The results suggest that while high SPF sunscreen may be unnecessary for short exposure, they may offer superior protection for longer exposure to the sun.

1959 Pimavanserin Tartrate, a Novel Anti-Parkinson Drug Suppresses Pancreatic Tumor Growth by Inhibiting Akt/Gli-1 Signaling Axis

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Despite major advances in cancer treatment, pancreatic cancer is still incurable and the treatment outcomes are limited. In the current study, we evaluated the anti-cancer effects of pimavanserin tartrate (PVT), an anti-parkinson drug used for the treatment of Parkinson’s disease psychosis. Our observations indicated that, PVT significantly suppressed the proliferation of pancreatic cancer cells by inducing apoptosis. Anti-proliferative and apoptosis inducing effects of PVT were mediated by the inhibition of pAkt (Ser473), Akt, Gli-1, Oct-4, Sox-2, Nanog, and c-Myc. Akt and Oct-4 inhibition by PVT treatment was further validated by immunofluorescence analysis. Apoptotic effects of PVT was confirmed by increase in cleavage of caspase-3 and PARP. In addition, PVT inhibited the formation of tumourspheres in PANC1 pancreatic cancer cells. Inhibition or silencing of Akt and Gli-1 using specific inhibitors or siRNA enhanced the growth suppressive effects of PVT in pancreatic cancer cells. Oral administration of PVT suppressed BxPC3 tumor xenografts by 50% in athymic nude mice. In another in vivo experiment, PVT treatment inhibited the growth of orthotopically implanted PANC1 tumors by 75%. Chronic administration of PVT did not exhibit any general signs of toxicity or behavioral side effects in mice. Since, PVT is already available in clinic with an established safety profile, our results will accelerate its clinical development for treatment of patients with pancreatic cancer. Taken together, our results indicate that pancreatic tumor growth suppression by PVT is orchestrated by inhibition of Akt/Gli-1 signaling.

1960 Design, Synthesis, and Evaluation of Novel Penfluridol Analogs with Optimized Toxicity Profile

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Penfluridol (PFL) is an antipsychotic agent that was used in the treatment of chronic schizophrenia since 1968. In the late 1980s, P. B. Mortensen has explored the potential use of PFL as an antineoplastic agent. Later, several groups have confirmed cytotoxicity of this drug in a variety of in vitro and in vivo cancer models, supporting an idea of the potential use of PFL in the treatment of cancer patients. However, monitoring of likely central nervous system (CNS) related extrapyramidal side effects was not performed. Majority of reported anticancer studies identified 10 mg/kg dosing as the most relevant for observing the desired effect. We have established that a 10 mg/kg dose of PFL (i.p. or oral) corresponds to a level of 1 µM of this compound in the brain. Such concentration is enough to block a majority of CNS receptors, including serotonin (Kd = 4.8, 5.6 µM) and PF331 (Kd = 4.8, 5.6 µM) and PF331 (Kd = 4.8, 5.6 µM) receptors and elicit unwanted side effects in patients undergoing chemotherapy. Thus, the use of PFL as an anticancer agent can be complicated by its ability to interact with CNS receptors. We proposed to eliminate the CNS toxicity of PFL by previously reported differences in structural requirements for its antipsychotic and anticancer properties. Design and synthesis of analogs were followed by in vitro evaluation of compounds using MTT assay in MDA MB 231 and LLC luc cell lines and by assessing the corresponding CNS receptors (major sub classes of dopamine, serotonin, sigma, opioids, and histamine) and transporters (DAT, NET, and SERT) binding profile (percentage of inhibition at 10 µM and Kd, values). As a result, we were able to identify compounds PF131 (IC50 = 4.8, 5.6 µM) and PF331 (IC50 = 5.4, 2.7 µM) with a substantial reduction in affinity towards CNS receptors and transporters when compared to PFL. Further, we have evaluated the toxicity profile (change in body weight, vital organ weight and clinical chemistry analysis of blood samples) of these compounds at the end of 7 days of treatment and assessed their distribution profile (C57BL6 female mice, i.p. dosing of 10 mg/kg, 0-96 hours). Finally, the anticancer activity of compound PF331 was analyzed in vivo using lung cancer model (C57BL6, i.p. daily dose of 10mg/kg) where 80% tumor size reduction was observed. In conclusion, we have identified a novel analog of PFL with improved toxicity profile and ability to reduce tumor size by 80% in vivo.
1961 Inhibition of Endothelial RhoA-ROCK Pathway Blocks Cancer Metastasis

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The principal health complication of cancer patients is metastasis: the disemination of cancer cells from the primary tumor to distant parts of the body. It is the cause of more than 90% of cancer-related deaths and the main goal in cancer research. An important step in metastatic process is the migration of the disseminating cancer cells through the endothelial monolayer during entrance or exit from the vasculature. The molecular mechanisms underlyng metastasis, especially the interaction of cancer cells with the endothelial lining of the vasculature is poorly understood. It has been shown that endothelial RhoA-Rho kinase (ROCK) pathway is responsible for vascular permeability through cytoskeletal remodeling. In the present study we investigated the role of endothelial RhoA-ROCK pathway in cancer cell trans-endothelial migration and eventually cancer metastasis. We have used a quantifiable, highly reproducible, transwell-based, two-cell co-culture model of trans-endothelial migration, where Green Fluorescent Protein (GFP) or Red Fluorescent Protein (RFP) expressing cancer cells transmigrate through an endothelial monolayer. Endothelial RhoA and the downstream signaling pathway were blocked by siRNA or pharmacological inhibitors. Biochemical assays were performed to identify the molecular mechanisms of cancer cell-induced RhoA activation in the endothelial cells. In vivo, endothelial specific RhoA-deficient mice were used in experimental metastasis models and Fasudil, a clinically relevant inhibitor of the RhoA pathway was utilized in wild-type mice to evaluate the clinical potential of our findings. A variety of different cancer cell lines of both murine and human origin were able to potentiate ROCK activation in the endothelial cells. Zt/g4-MMAE at 10 mg/kg in a Q12 x 2 regimen completely eradicated TNBC subpopulation and reduced sphere formation of TNBC stem-like cells.

1962 RON Receptor-Targeted Antibody-Drug Conjugate Therapy Eliminates Cancer Stem-Like Cells and Induces Long-Term Tumor Regressions in Preclinical Models of Triple-Negative Breast Cancer (TNBC)

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Triple-negative breast cancer (TNBC) is a highly diverse group of malignant neoplasia with poor outcome. Currently, the lack of effective targeted therapy has fostered a major effort to discover new targets to treat this malignant cancer. Here we identified the RON receptor tyrosine kinase as a therapeutic target for potential TNBC treatment by using a novel anti-RON monoclonal antibody (Zt/g4- anti-RON (MMAE)) Anti-RON antibody Zt/g4 was conjugated through a dipeptide linker to MMAE, a potent tubulin inhibitor. The generated Zt/g4-MMAE has a drug-antibody ratio of 3.2:1. The conjugation profile and drug stability were analyzed using HPLC. A panel of TNBC cell lines representing different subtypes and expressing different levels of RON was used as a biological model. Zt/g4-MMAE-induced cell surface RON internalization, cell-cycle arrest, and cellular cytotoxicity. We first analyzed RON expression in 168 primary TNBC samples via tissue microarray using anti-RON IHC and demonstrated that RON was widely expressed in 76.8% TNBC samples with overexpression in 76.2% of cases (45.2%). These findings provide the basis to target RON for TNBC therapy using anti-RON ADC (Zt/g4-MMAE). In vitro, Zt/g4-MMAE rapidly induced RON internalization, resulted in cell cycle arrest followed by massive cell death. Zt/g4-MMAE also effectively killed TNBC stem-like cells with RON+/CD44+/CD24- phenotypes and RON-negative TNBC cells through the bystander effect, which effectively eliminated CSC subpopulations and reduced sphere formation of TNBC stem-like cells. In vivo, Zt/g4-MMAE at 10 mg/kg in a Q12 x 2 regimen completely eradicated TNBC xenografts caused by two TNBC cell lines without the regrowth of xenograft tumors. Increased RON expression is a pathogenic feature in primary TNBC samples. Zt/g4-MMAE is highly effective in eradicating TNBC xenografts in preclinical studies with lasting effects up to 6 weeks without signs of tumor regrowth. The current study has met our objectives. The first was to determine levels of RON expression from primary TNBC samples by anti-RON immunohistochemical (IHC) staining. The second was to validate the efficacy of anti-RON antibody Zt/g4-drug monomethyl auristatin E (MMAE) conjugate for TNBC therapy. These studies provide the basis for targeting RON as a novel strategy for TNBC treatment using anti-RON ADC Zt/g4-MMAE.

1963 Evaluation of the Potential Carcinogenicity of the Pyrethroid Imiprofin in Mice

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The carcinogenic potential of the non-genotoxic pyrethroid imiprofin was examined in two long-term bioassays, one in SD rats and the other in CD-1 mice. There was no carcinogenic activity in rats up to 5000 ppm of the diet. In mice, imiprofin was administered to males and females at dietary concentrations of 0, 100, 3500, or 7000 ppm, with a reduction of body weight at 7000 ppm indicating systemic toxicity. The Maximum Tolerated Dose (MTD) was exceeded at 7000 ppm for both males and females (body weight reductions of 15% and 22% versus controls, respectively). In addition, significant increases in mortality were seen in females in the 7000 ppm group (45.1%, treated vs. 13.7%, controls). Under these conditions, there was a higher incidence of lung adenocarcinomas in the 7000 ppm male group that was statistically significant (p=0.03) by a Fisher’s exact test. However, the most widely used procedure to adjust for multiple comparisons for commonly occurring tumors (including lung tumors in the male mice) requires p<0.01 statistical significance for a pairwise comparison, so by this criterion the increase is not statistically significant. Moreover, there were no significant increases (even at the p<0.05 level) in the incidences of combined adenomas and adenocarcinomas, the most appropriate evaluation, in any dose group, male or female. There were significant (p<0.05) trends for increased adenomas and combined adenomas and carcinomas with increased dose in females and for adenocarcinomas in males, using the Cochran-Armitage linear trend test and Poly-3 test. However, if the highest dose is removed from consideration owing to its exceeding the MTD, there are no significant increases (trends or pairwise comparisons) in tumor formation. Additional evaluation of lung lesion development indicated that there were no statistically significant increases in any tumor formation in any dose group, even at p<0.05. Based on high susceptibility of this mouse strain for appearance of lung tumors and the lack of a statistically significant increase in tumors, the mouse study does not indicate a carcinogenic effect of imiprofin, and thus no classification for carcinogenicity is appropriate.

1964 Overcoming the Acquired Drug Resistance of Gefitinib in A549 via Downregulations of Twist1

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Overexpressed Twist1 in primary and metastatic non-small cell lung cancer (NSCLC) has been identified as a critical therapeutic target. Our previous study has demonstrated that Twist1 was significantly increased in gefitinib-resistant A549 (A549GR) compared to A549, which could play a critical role in the epithelial mesenchymal transition (EMT) observed in A549GR. Our present study showed that compared with A549, A549GR was also resistant to AZD9291, a third generation of epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI). Knockdown of Twist1 in A549GR was carried out using Twist1 shRNA plasmid (A549GR-KO-T). Significantly increased sensitivity to gefitinib or AZD9291 was observed in A549GR-KO-T compared to A549GR and/or A549GR transfected with scrambled control plasmid (A549GR-KO-C). The IC50s (µM) of A549GR and A549GR-KO-C to gefitinib was 51±1.0 and 50±1.6, respectively, while the IC50 of A549GR-KO-T to gefitinib was 35±2.0. The IC50s (µM) of A549GR, A549GR-KO-C, and A549GR-KO-T to AZD9291 were 12.0±1.0, 11.8±0.7, and 8.3±0.6, respectively. Moreover, it was found that knockdown of Twist1 could significantly enhance the EGFR-TKI therapeutic effects in lung cancer cells with acquired EGFR-TKI resistance.
Among the mechanisms of gene expression regulation involving posttranslational modification of messenger RNA, adenosine N6 methylation (m6A), is the most prevalent. This modification participates in a variety of RNA processing, including splicing, mRNA stabilization as well as in translation regulation. Interestingly, the gene expression associated with stem cell pluripotency and specific differentiation are controlled by m6A levels on mRNAs. The decrease in the m6A mark due to the increased action of the demethylases FTO / ALKBH5 or to a defect on methyltransferases (MTs), leads to the acquisition of cancer stem cell (CSC) markers (CD44, SOX2 and NANOG) and is often linked with cancer development. Exposure to inorganic arsenic is associated with prostate cancer in humans and in vitro cellular models exposed to this toxicant show an increased expression of CSC markers. This observation suggests that in these cells the mechanism associated with m6A RNAs regulation should be altered and could play a role in the malignant transformation of prostate CSCs. In this work we evaluated the demethylases FTO and ALKBH5 and the MTs MTTL3 / MTTL14 transcript levels, as well as stem cell markers CD44, NANOG and SOX2 gene expression in prostate CSCs malignantly transformed with sodium arsenite (As-CSCs). These cells showed a significant over-expression of FTO (p=0.0174) and an increased expression of ALKBH5 (p=0.0772) that correlated with a significant increase in the expression of SOX2 (p=0.0002) and the 5 cmarker CD44 (p=0.0011). These results suggest that inorganic arsenic may favor the selective growth of CSCs that over-express SOX2 as a consequence of an increased removal of the m6A mark in mRNAs that code for this gene; such events may favor the development of cancer in prostate cells.

### 1965
**Arsenic Malignantly Transformed Prostate Cancer Stem Cells Show Increased FTO/ALKBH5, Which Is Associated with Increased SOX2 and CD44 Transcripts Levels**


### 1966
**A Case Study Using a Systematic Review Approach for Cancer Hazard Identification That Incorporates the 10 Key Characteristics of Carcinogens**


Evaluating the carcinogenic mechanisms for a substance is challenging, in part due to the broad range of information and lack of a widely accepted approach. The 10 key characteristics of carcinogens (KCs) offer a way to identify and organize mechanistic information. Systematic review (SR), a review approach particularly useful in handling inconsistent results, aims to answer a specific question while minimizing bias by using a pre-defined protocol to search, evaluate, and synthesize all relevant studies for the question. We used KCs and an SR approach to synthesize cancer mechanistic information of antimyoxid (Sb(O)3), a synergist for flame retardants and catalyst in polyethylene terephthalate (PET) plastics production, along with selected other compounds containing trivalent antimony. First, references were identified in three databases using a systematic literature search strategy and selected with predefined inclusion and exclusion criteria. Second, mechanistic studies were evaluated individually by at least two scientists for quality and for utility to inform mechanisms of Sb(O)3 carcinogenicity. Factors relating to test article (e.g., purity), model system (e.g., animals, cell lines, bacterial strains), method (e.g., consistency with current guidelines for in vitro human cells and spem whale skin cells). We compared these results to data from human cell studies. Whales are our closest marine relatives, have long lifespans, breathe air, and are exposed to environmental Cr(VI). Importantly, they have low cancer rates and cell culture studies indicated that Cr(VI) induced aneuploidy and centrosome amplification in whale cells and compare to data from human cell studies. Whales are our closest marine relatives, have long lifespans, breathe air, and are exposed to environmental Cr(VI). Importantly, they have low cancer rates and cell culture studies show they are resistant to Cr(VI) genotoxicity. Thus, we investigated Cr(VI)-induced aneuploidy and centrosome amplification in sperm whale lung cells and compare to data from human lung cells. Fibroblasts from human lung, bowhead whale lung, and sperm whale skin were treated with zinc chromate, a particulate Cr(VI) compound. Aneuploidy was measured by analyzing solid stained chromosomes. Zinc chromate exposure for 24 h did not cause aneuploidy changes in any cell line. In human cells, 120 h exposure to 0.1, 0.2, 0.3 and 0.4 µg/cm² zinc chromate induced aneuploidy in 13.5%, 17.6%, 23.4%, 25.4% and 36.5% of cells. However, while both cell lines retained low rates of aneuploidy, spindle assembly checkpoint (SAC) bypass can cause aneuploidy by allowing chromosomes to dissociate prior to proper kinetochore attachments. Evidence of SAC bypass was measured via solid stained chromosomes and 120 h treatment of 0.2, 0.3 and 0.4 µg/cm² zinc chromate induced centromere spreading, premature centromere division and premature anaphase in human cells. No increase in SAC bypass was observed in whale cells at any treatment concentration or time point. Supernumerary centromeres can cause CIN through multiple mitoses and asymmetrical chromosomal segregation. Fluorescently immunostained centromeres were counted after Cr(VI) treatment. Centrosome amplification was unchanged after 24 h exposure in all cell lines, but at 120 h increased in human cells by 9-16.4% in interphase and 18-43% in mitotic cells, while whale cells exhibited no increases. These data

### 1967
**Improving Formalin-Mixed Paraffin-Embedded (FFPE) Samples for DNA-Sequencing Analysis**

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Archival FFPE samples provide an untapped resource for mining genomic data. However, damage of nucleic acids during formalin fixation leads to latent sequencing artifacts. Previously, we showed that organocatalyst use during RNA isolation protocols improved FFPE RNA-sequencing data. The goal of the current study is to evaluate whether (a) a novel organocatalyst treatment improves FFPE DNA-seq data, (b) RNA-seq variants can be filtered out formalin-induced sequence artifacts in DNA, and (c) time in formalin alters these FFPE DNA-seq analyses. Here, we isolated RNA and DNA +/- organocatalyst from paired FFPE and frozen human kidney and ovarian cancer specimens collected through the Biospecimen Pre-analytical Variables Program-National Cancer Institute. Sample collection/processing was prospectively designed to evaluate common pre-analytical variables. Both renal and ovarian carcina samples were either frozen and embedded in OCT or formalin-fixed for 12 hours prior to paraffin embedding. Ovarian carcinoma samples also included a 72-hour formalin fixation timepoint. Tumor types were microscopically verified and nuclei counts were obtained (Aperio Slide Scan). For nucleic acid normalization and positively associated with RNA and DNA yields (r= 0.142 and 0.383, respectively). RNA and DNA were isolated +/- extended organocatalyst incubation (Qiagen FFPE AllPrep). Total RNA-seq library preparation required 4 to 6 FFPE curls 10 µM thick with more for FFPE DNA where yields were 1.6-16.4-fold higher. Formalin-fixed samples had ~1.8/4.5-fold less RNA/DNA, respectively compared to the 12 hr. formalin-fixed samples (4.7 ± 2.6/1.1 ± 0.9 µg). Total RNA and DNA-exome sequencing were performed by Q2 solutions (Durham, NC). Across FFPE samples, we identified ~2-fold higher reads mapped to intronic regions (50-60% total) and half the reads mapped to exons (25-30%) vs. paired frozen. While use of the organocatalyst improved FFPE RNA yields, it did not with the matching FFPE DNA. This suggests that incubation conditions ideal for reversing formaldehyde damage in FFPE RNA may not be the same as in FFPE DNA. With the growing importance of large-scale genomic analyses, this work will have important applications in mechanistic toxicology and precision medicine. This study does not reflect US EPA policy.

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support the hypothesis in comparison to human lung fibroblasts, whale cells have cellular strategies to protect against Cr(VI)-induced centrosome amplification, SAC bypass, and numerical chromosome instability. This work was supported by NIHES grant R01ES016892 (J.P.W.).

1969 Nuclear Receptor 4A1 (NR4A1) as a Drug Target for Endometriosis

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Endometriosis is a common but complex inflammatory disease that primarily affects women during their reproductive years, and it is estimated that 5,500,000 women in the United States and 176,000,000 worldwide exhibit symptoms of endometriosis. Current therapies for endometriosis include progestins, oral contraceptives and GnRH antagonists however since these therapies are hormonal these treatments are not viable for women of reproductive age and there is an urgent need for development of new and more effective therapies. The orphan receptor NR4A1 is expressed in cancer and in endometriotic tissue and ongoing studies in this laboratory demonstrate that 1,1-bis(3′-indolyl)-1-(p-hydroxyphenyl) methane (DIM-C-pPhOH) and related compounds act as NR4A1 antagonists and inhibit NR4A1 regulated pro-oncogenic pathways. In this study we have used patient-derived endometriotic cells and normal endometrial cells and DIM-C-pPhOH inhibited growth and induced apoptosis in endometriotic but not in normal cells. Moreover we also observed similar results in cells transfected with oligonucleotides targeting NR4A1 (siNR4A1) demonstrating an important role for NR4A1 in endometriotic cell growth and survival and this was also confirmed in an in vivo model of endometriosis in mice. Moreover, we also observed that specific pathways (eg. mTOR) and genes (survivin and epidermal growth factor receptor (EGFR)) that are well characterized pro-oncogenic factors are also regulated by NR4A1 and treatment by with the NR4A1 antagonist DIM-C-pPhOH or knockdown of NR4A1 by RNA interference inhibited mTOR signaling and decreased expression of survivin and EGFR. These new data suggest that NR4A1 antagonist are novel mechanism-based agents for treating endometriosis.

1970 Definition of Biological Thresholds for the Identification and Mode of Action Classification of Tumorigenic Chemicals


Chemicals induce liver cancer in rodents largely through well-characterized adverse outcome pathways (AOPs). In this study we defined biological thresholds for molecular initiating events (MIEs) and downstream key events (KEs) in liver cancer AOPs in short-term assays. We used the rat in vivo TG-GATES study data to measure MIEs (genotoxicity, cytotoxicity, and activation of AhR, CAR, ER, and PPARα) and associated KEs (cell hypertrophy/proliferation assessed indirectly by liver to body weights) across 77 chemicals that could be linked to doses with previously established effects on rat liver tumor induction. The genotoxicity biomarker was comprised of 7 p53-responsive genes known to be induced upon DNA damage. Gene expression biomarkers for receptor-based MIEs (AhR, CAR, ER, and PPARα) were built using microarray comparisons from the livers of rats treated with prototypical activators. Thresholds for genomic biomarkers were determined by first calculating the significance of the correlation between a biomarker and each filtered gene list derived from a microarray analysis of each chemical-dose-time exposure. For the entire dataset of >1000 comparisons, this analysis was followed by a Haber transformation, linear regression, and calculation of the 99% confidence interval for the -log(p-value) = 4-12). The genomic biomarker thresholds from an independent dataset of microarray data (DrugMatrix) derived using the same methods were very similar to those developed from TG-GATES, demonstrating consistency across chemical sets and microarray platforms. Additionally, chemical-independent thresholds for liver to body weights (~20% increase), ALT (~80% increase), and AST (~100% increase) were defined using similar approaches. The thresholds for the genomic biomarkers derived from the TG-GATES data could predict not only liver tumor induction in the TG-GATES study (balanced accuracy = 96%) but also the DrugMatrix study (balanced accuracy = 92%). The biological thresholds defined in this study could be used to predict tumorigenic potential in short-term studies and determine the mode of action underlying the tumors. This abstract does not represent US EPA policy.

1971 Platinum Leaving Ligand Effects on Mammalian Cells

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Cisplatin, Carboplatin, and Oxaliplatin are the three US FDA approved platinum-based chemotherapy agents. Each compound is defined by two ligands attached to a central platinum atom. Intracellularly, the nonleaving ligands remain attached to the central atom. However, the leaving ligands are released from the platinum and allow the platinum to bind to cellular components such as DNA. It is the coordination of the platinum atom with the attached nonleaving ligand to DNA that induces apoptosis, the primary mechanism of death for these compounds. Compound structure correlates with compound efficacy as a cancer treatment. For example, Cisplatin and Carboplatin have similar nonleaving ligands and are approved by the US FDA for treatment in many of the same cancer types. Oxaliplatin is a structurally distinct compound from Cisplatin and Carboplatin and is approved to treat cancers arising from different tissues. This project compares cell-specific toxicity of novel platinum compounds in both cancerous and noncancerous cell lines to better understand the role of structure in cytotoxicity. We tested two novel compounds, Dichloro[ethylenediamine]platinum(II), abbreviated as Pt(en)Cl2, which differs from Cisplatin only in the nonleaving ligand, and Oxalato(S,R,RS)-N,N'-dimethyl-1,2-diaminocyclohexaneplatinum(II), abbreviated as Pt(Me2dach)ox, which differs in both ligands. Pt(en)Cl2, was tested in both cancerous (NTera-2) and noncancerous cell lines (HEK293) and preliminary data shows higher toxicity in cancerous cells lines versus noncancerous lines. Current data indicates an IC50 ~ 90µM for Pt(en)Cl2, in control HEK293 cells.

1972 Nuclear Receptor 4A2 (NR4A2) as a Drug Target for Treating Glioblastoma

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The orphan nuclear receptor 4A2 (Nurr1) is a member of a family of receptors with no known endogenous ligands and was initially identified as a rapidly induced gene in cells under stress. NR4A2 has been extensively characterized in subcellular regions in the brain, and NR4A2+ mice do not generate midbrain dopaminergic neurons and die soon after birth. Studies in several laboratories have been investigating the role of NR4A2 in Parkinson’s disease, and our collaborative research has focused on the effects of 1,1-Bis(3′-indolyl)-1-(p-chlorophenyl)methane (DIM-C-pPhCl (CDIM 12)) on animal models of Parkinson’s disease. We have hypothesized that NR4A2 may also play a role in another neuronal-derived disease, namely glioblastoma multiforme (GBM) and our preliminary studies show that NR4A2 is overexpressed in a unique set of patient derived glioblastoma (GBM) cells (14015s, 14104s, 15037, 15049 and 17008). We used anti sense oligonucleotides (FANA molecules) to knockdown NR4A2 to investigate the roles of this receptor in patient derived glioblastoma cells. Knockdown of NR4A2 in 14015s and 15037 cells decreased cell proliferation, decreased migration/invasion and induced Annexin V staining (apoptosis) and these functional responses were accompanied by downregulation of several genes (survivin, Bcl2, cMet, EGFR, Q5 integrin and β3 integrin) and induction of cleaved caspase-3 and PARP indicative of apoptosis. Our results showed that knockdown of NR4A2 and 1,1-Bis(3′-indolyl)-1-(p-chlorophenyl)methane DIM-C-pPhCl (CDIM 12) is an NR4A2 ligand previously investigated in our laboratory that decreased NR4A2-dependent transactivation in 14015s and 15037 cells and inhibited cell growth, migration/invasion and induced apoptosis thus acting as an NR4A2 antagonist/reverse agonist in GBM cells. Moreover, the effects of NR4A2 knockdown and the C-DIM-NR4A2 antagonist were comparable as inhibitors of NR4A2-dependent genes/pathways. These preliminary results from NR4A2 knockdown studies and treatment with an NR4A2 antagonist demonstrate for the first time that NR4A2 is pro-oncogenic in GBM and can be targeted by NR4A2 antagonists, such as DIM-C-pPhCl and related compounds. Ongoing studies are focused on investigating the mechanism of action of NR4A2 antagonists as a new class inhibitors of GBM and developing a series of more potent analogs for clinical applications in treating this deadly disease.

1973 Using the Key Characteristics of Carcinogens in Carcinogen Hazard Identification

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Smith et al. (Env. Health Perspect. 124: 713, 2016) identified 10 key characteristics, one or more of which are commonly exhibited by established human carcinogens. The key characteristics of carcinogens (KCs) reflect the chemical
and biological properties of cancer-causing agents and are distinct from the hallmarks of cancer, which are the properties of tumors. The KCs have been applied in evaluating mechanistic data for more than 30 diverse agents in recent IARC Monograph meetings. The Group 1 and 2A carcinogens identified in these meetings exhibited a range of KCs, including 'is genotoxic', 'is immunosuppressive' or 'modulates receptor-mediated effects', in some cases in combination with other KCs. For most Group 2A and 2B agents, fewer studies relevant to the KCs were identified. Because the KCs are based on empirical observations of characteristics associated with known carcinogens, they provide an agnostic and unbiased survey of the mechanistic literature. This improved uniformity across evaluations, revealing strengths as well as gaps in evidence. A focus on STAT3 signaling was chosen because it is a hallmark of many cancers. Further, this approach also has the advantage of being non-specific to particular disease subtype.

For each agent, the following data were curated:

- The exposure details: These included the chemical exposure concentrations as well as relevance to human exposure.
- The in vivo and in vitro models used.
- The primary endpoints assessed and the results.
- The mechanistic data related to the KCs.

The data were then used to develop a comprehensive understanding of the molecular mechanisms underlying the carcinogenic effects of the agents considered. A molecular signature was developed for each agent that reflects the relevant biological properties and is distinct from the KCs.

1976 Uterine Adenocarcinomas in Isopryrazam-Treated Rats Occur via a Human Non-relevant Mode of Action

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Female rats treated with 3000 ppm (232.8 mg/kg BW/day) isopryrazam (IZM) for 2 years had a greater incidence of uterine adenocarcinomas, lesser incidence of pituitary and mammary tumors, and ~40% reduction in body weight (BW) gain. For this investigational study, female Han Wistar rats were treated with 0, 500 and 3000 ppm IZM (28 and 194 mg/kg BW/day, respectively) for up to 18 months. Rats were euthanized and necropsied at 13, 26, 52, 66 and 80 weeks. Blood was collected at 4, 13, 26, 52, 66 and 80 weeks for measurement of prolactin and leptin. Selected tissues were weighed, and histology of the influenced by dietary intake and may regulate pro-inflammatory adipokine secretion. Daily vaginal lavages were performed for 2-3 week intervals throughout the study. Decreased fat pad weights, increased liver weights and reduced BW gain followed a dose-responsive pattern. The high dose group cyclically regulated for a longer period of time and had a greater percentage of rats in persistent estrus from weeks 52 to 80 compared to control. Prolactin and leptin levels were significantly less in the high dose group, and dopamine signaling to the pituitary was greater than controls at ≥26 weeks. Based on the data, a mode of action (MoA) is established that begins with decreased food utilization, fat pad weight and body weight, reflected by the decreased leptin levels. As a consequence, there is altered hypothalamic signaling that results in altered pattern of transition into reproductive senescence. The longer time spent in persistent estrus and greater exposure to circulating estrogens leads to the observed increase in uterine adenocarcinomas. Fundamental differences exist between rats and humans in the control of reproductive senescence. The onset of senescence in rats is due to altered signaling within the hypothalamus, while menopause and reproductive senescence in humans is driven by depletion of a set number of primordial follicles in the ovaries with aging. The physiological difference in control of the reproductive cycle and the transition into reproductive senescence between rats and humans indicate that the established MoA leading to uterine tumor formation in rats is not relevant to humans.
Efficient Carcinogenesis via Mutated Inflammation-Activated Stem Cells: A New Theory Explains Why Nrf2-Activation Blocks Aflatoxin-Induced Liver Cancer in Rats

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Jones et al. (Cancer Prev Res 2014; 7(7):658-65) recently showed that ~100% incidence hepatocellular carcinoma (HCC), by week 104 in male rats dosed by daily gavage with aflatoxin B, (AFB, ) and F for weeks starting at week 6, could be blocked completely—but without similar elimination of AFB-DNA adducts—by co-administering a highly potent anti-inflammatory Nrf2 activator (CDDO-im) for 5 weeks starting at week 5. Time-to-tumor data for rats in that study treated only with AFB, are clearly consistent with a 2-stage doubly stochastic multistage somatic mutation (MSM) cancer model. However, after reducing multiple experimental errors proportional to AFB-DNA adduct reductions measured during/after CDDO-im co-treatment, that MSM model predicts an HCC incidence rate (46%) far greater than was observed (0/20, 2-tail p = 10^{-5} assuming binomially distributed error). A novel alternative theory explains the combined study data: Cancer arises most efficiently in epithelial tissues from just any stem cell with critical mutations, but only from a stem cell that has or will become activated to an epigenetically mediated and maintained state of adaptive/regenerative hyperplasia—a state normally triggered by immune cells that generate and coordinate local inflammation at the site of tissue injury. Normally such inflammation-activated stem cells (IASCs) are relatively rare, because they arise transiently as part of a highly orchestrated process of inflammation and tissue repair tightly restricted to the site of injury. However, the new theory posits that IASCs are each uniquely susceptible to efficient carcinogenesis involving as few as two critical somatic mutations, provided the new theory posits that IASCs are each uniquely susceptible to efficient carcinogenesis involving as few as two critical somatic mutations, provided the new theory posits that IASCs are each uniquely susceptible to efficient carcinogenesis involving as few as two critical somatic mutations, provided the new theory posits that IASCs are each uniquely susceptible to efficient carcinogenesis involving as few as two critical somatic mutations, provided the new theory posits that IASCs are each uniquely susceptible to efficient carcinogenesis involving as few as two critical somatic mutations. A key feature of this inflamed stem cell is its likelihood status as an inflammation-activated stem cell (IASC) that, upon activation, becomes a constitutive and permanent powerful effector of cancer progression.

Mechanistic Role of Cytochrome P450 1 Enzymes in Polycyclic Aromatic Hydrocarbon Mediated Carcinogenesis

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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. Cytochrome P450 (CYPs) enzymes are known to 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 mice lacking Cyp1 expression will be resistant to polycyclic aromatic hydrocarbon-mediated pulmonary carcinogenesis. Two-month-old A/J (WT) and Cyp1a1/1a2/1b1-triple knockout (Cyp-1-3ko) mice were exposed to 3-methylcholanthrene (MC; 100μmol/kg), benzo(a)pyrene (BP; 200 μmol/kg), or corn oil (vehicle) via a single intraperitoneal injection. Liver and lung tissues were harvested at 1, 8, and 15 days post PAH exposure for our short-term studies. Mice in our tumor study were harvested 36 weeks post PAH exposure for tumor incidence and multiplicity analysis. We found that MC caused significant induction of hepatic CYP1A1/A2 gene expression in WT but not Cyp1-3ko mice at each time point. MC treatment caused formation of DNA adducts in liver and lung for up to 15 days post MC treatment in both genotypes. However, compared to WT animals, Cyp-1-3ko mice had significantly reduced hepatic DNA adducts on day 15 and up to 95% less pulmonary DNA adducts at 1, 8 and 15 days post MC treatment. In addition to the DNA adduct inhibition, Cyp-1-3ko mice formed 93% less pulmonary tumors compared to the MC-treated WT counterparts. The significant reduction in DNA adducts and pulmonary tumors in Cyp-1-3ko mice provide strong evidence of the mechanistic role these enzymes play in PAH-mediated carcinogenesis. The mechanistic insight our study provides will be tremendously important for finding preventative and therapeutic avenues to combat PAH-mediated carcinogenesis.

Synthetic Progestins Elicits Similar Proliferative and Gene Expression Responses as Endogenous Progesterone at Much Lower Concentrations

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The use of the synthetic progestin, medroxyprogesterone acetate (MPA), during hormone replacement therapy (HRT) has been linked to increased risk of invasive breast cancer. Currently, the biological mechanism underlying this relationship is unknown. Synthetic progestins are a class of pharmaceutical compounds that bind the progesterone receptor and are predominantly used as birth control and during HRT. It is unclear which commonly used synthetic progestins, besides MPA are associated with invasive breast cancer. The goal of this study is to understand whether breast epithelial cells have the same physiological and transcriptional response to endogenous progesterone and synthetic progestins. T47D cells, a breast epithelial cell line, were grown in culture and exposed to endogenous progesterone (P4) and four common progestins: (1) MPA, (2) etonogestrel (ET), (3) norethindrone (NE), and (4) levonorgestrel. First, a dose course was conducted to determine the effects on cell proliferation, as growth arrest is a hallmark of progesterone exposure. Cells were exposed at physiologically relevant concentrations of P4 (0.2-700nM) and the four progestins (0.2-20nM). Next, gene expression changes were assayed at physiologically relevant doses for both P4 (200nM) and the progestins (2nM). To determine transcriptional responses to progestins, we profiled mRNA levels. Assessment of the biological fate of HT-29 cells overexpressing GLTP demonstrated that GLTP overexpression modulated cell shape change but the magnitude of change differed greatly, with induction of cell death (e.g., in >150nM), as well as the progesterone response sentinel genes, Dual Specificity Phosphatase 1 (DUSP1) and Zinc Finger And BTB Domain Containing 16 (ZBTB16) were assayed across the time points 2, 4, 6, 8, 10, 12 and 24 hours. Gene expression responses peaked between 10-24 hours for all compounds. However, the magnitude of change differed greatly, with induction of ZBTB16 and DUSP1 around 20-fold, while PGR was induced over 200-fold. These results demonstrate that progestin compounds are able to elicit similar proliferative and gene expression responses as P4 at much lower concentrations. Future work will focus on transcriptome level differences between P4 and progestins.

Glycolipid Transfer Protein (GLTP) Regulates Non-apoptotic Cell Death in Colon Cancer Cells: Implications in Cancer Therapy and Cytotoxicity

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Sphingolipids are known to affect cancer progression by regulating cell death (apoptosis) and survival (autophagy). Whereas apoptosis is promoted by elevations in sphingosine and ceramide, increases in sphingosine-1-phosphate and ceramide-1-phosphate can tip the balance toward survival. Human glycolipid transfer protein (GLTP) is a small (24 kDa) amphipathic protein encoded on chromosome 12 (locus 12q24.11) that can mediate non-vesicular transport of metabolic enzymes in the Golgi apparatus. Changes in the expression of GLTP has been shown to modulate cell shape change but the effect on regulating cell proliferation and cancer progression remains unexplored. We herein show that GLTP overexpression inhibits the growth of human colon carcinoma cells (HT-29; HCT-116), but spares normal colonic cells (CCD-18Co) largely due to growth arrest at the G0/G1 cell cycle checkpoint. Mechanistically, we found that GLTP overexpression modulated the cell cycle progression of upregulating Kip1/p27 and Cip1/p21 protein and mRNA levels, while decreasing CDK2, CDK4, cyclin E and cyclin D1 protein levels. Assessment of the biological fate of HT-29 cells overexpressing GLTP revealed increased levels of phosphoeylated human mixed lineage kinase domain-like (MLKL) protein, intracellular calcium and necrotic cell death. Overexpression of W96A-GLTP, a mutant incapable of transferring GSLs, failed...
1983 Comprehensive Molecular Characterization of Mitochondrial Genomes in Spontaneous and Chemical-Induced Hepatocellular Carcinomas in B6C3F1/N Mice

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Mitochondria play an important role in cellular energy metabolism. Under xenobiotic stress, free radical generation and subsequent chronic oxidative stress have been implicated in many carcinogenic processes. The origin and impact of mitochondrial DNA (mtDNA) alterations in chemical induced carcinogenesis are unclear. We performed ultra-deep whole mtDNA sequencing, whole exome sequencing and mtDNA copy number analysis on the fresh-frozen B6C3F1/N male hepatocellular carcinomas (HCCs) that arose either spontaneously (n=10) or due to 2-year exposure to a genotoxic carcinogen, gingko biloba extract (GBE; n=10) and a non-genotoxic carcinogen, antraquinone (n=10); age-matched non-tumor controls (n=10) were also included. In total, 25 somatic substitutions were detected in the mitochondrial genome. Mutation signature analysis of the mitochondrial genome demonstrated a similar pattern (A/C to G/T transitions) across all tumor samples. The mtDNA copy number analysis revealed a significant reduction in spontaneous (p=0.0017) and antraquinone-induced (p=0.0006) HCCs but not in GBE-induced HCCs (p=0.0356). The number of nuclear-encoded mitochondrial genes mutations in the GBE-exposed tumors was slightly higher (122 mutations per sample) compared to the number of mutations in the spontaneous (97.4 mutations per sample) or antraquinone-exposed (91.7 mutations per sample) tumors. Our findings indicate that the endogenous mutational mechanism has greater impact than any other external mutagens in mtDNA and is fundamentally linked to mtDNA replication. Exposure to GBE or antraquinone may have different mechanisms of action on mitochondrial dysfunction. Mutation signatures within mtDNA and nuDNA are distinct within the same tumor arising spontaneously or due to chemical exposure. However, mitochondrial copy number alterations and mutations in nuclear genome are altered by chemical exposures.

1984 Mechanistic Data Can Play a Pivotal Role in IARC Monographs Evaluations When Human Data Are Less Than Sufficient

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Based on an integrated evaluation of evidence for carcinogenicity in humans and experimental animals, and of carcinogenic mechanisms, the IARC Monographs identify environmental factors that can increase the risk of human cancer. Mechanistic evidence can aid in identifying carcinogens when human data is less than sufficient. Here we review important recent evaluations that were influenced by strong mechanistic evidence, and highlight types of evidence most influential in classifications of cancer hazard. In 1997, 2,3,7,8-tetrachlorodibenzo-p-dioxin was first classified in Group 1 (carcinogenic to humans) based on sufficient evidence in animals and mechanistic considerations, an evaluation later confirmed by sufficient evidence in exposed humans in 2012. More recently, tetrachloroazobenzene was classified as "probably carcinogenic to humans" (Group 2A) because it belongs, on the basis of mechanistic considerations, to this same class of agents. Furthermore, in 2016, an upgrade from Group 2B to Group 2A was warranted for tetrabromoethylene because it satisfies the mechanistic criterion for Group 2A. Our findings indicate that the endogenous mutational mechanism has greater impact than any other external mutagens in mtDNA and is fundamentally linked to mtDNA replication. Exposure to GBE or antraquinone may have different mechanisms of action on mitochondrial dysfunction. Mutation signatures within mtDNA and nuDNA are distinct within the same tumor arising spontaneously or due to chemical exposure. However, mitochondrial copy number alterations and mutations in nuclear genome are altered by chemical exposures.

1985 Zonal-Specific Transcriptional Programs Associated with PPARα Activation in the Rat Liver and Their Role in Liver Cancer in Rodents

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Previously, we compared whole genome gene expression following exposure to multiple doses of GW7647 - a ligand with high activity for PPARα - in rat and human hepatocytes and in the intact rat liver. Using ChIP methods, we found that the PPARα peroxisomal proliferator response element (PPRE) binding sites in human and rat hepatocytes differed qualitatively for down regulated genes in the rat. In addition, responses in the intact rat were more diverse than in rat hepatocytes with many more gene families significantly down regulated. We thus performed ultra deep sequencing, whole exome sequencing and mtDNA copy number analysis to assess the seven IARC criteria for an α2u-globulin-associated tumorigenic response. One (ethyl acrylate) caused fore-stomach tumors, but human relevance could not be ruled out because it did not lack genotoxic activity.
ences in the capacity of PPARα to cause proliferation, while the differential periporal responses affected by PPARα agonists may be a key step in cell proliferation and carcinogenesis associated with PPARα agonists in rodents.

1986 Application of ToxCast, ToxPi and Read-Across for Analyzing the Potential Carcinogenicity and Mutagenicity of Some Di- and Tri-phenylmethanes


Di-phenylmethanes are commonly used as intermediates in the production of dyes and pigments, and tri-phenylmethanes are often used as food additives or to dye textiles. There are more than a hundred chemicals in this group and most have not been tested for carcinogenicity or mutagenicity. To supplement the limited carcinogenicity and mutagenicity data for these chemicals, we took two approaches. First, we explored the use of high-throughput toxicity screening data available for chemicals in this group within the ToxCast Database (https://www.epa.gov/chemical-research/toxicity-forecasting), IARC’s key characteristics for carcinogens, and Toxicological Prioritization Index (ToxPi, http://toxpi.org/), a visual analytics tool, to predict carcinogenicity. Second, we applied a read-across tool, ToxRead (http://www.toxread.eu/index.php), to predict the mutagenicity of the chemicals in this group that had ToxCast data. We identified ToxCast data for 19 chemicals in this group. Six are di-phenylmethanes, and all but one are intermediates in the production of dyes. Thirteen are tri-phenylmethanes, all are dyes, and four are approved as food additives in either the US or the EU. In order to predict carcinogenicity, we aligned the active ToxCast Assays for each chemical to the key characteristics of carcinogens. ToxPi was then used to rank each chemical, based on level of activity in cancer pathway-related assays. Two of the nineteen chemicals (C.I. Basic Red 9 and Michler’s ketone) are known mutagens and carcinogens, and served as positive comparison chemicals. The results showed that several of the other chemicals share certain similarities in both ToxPi scores and slice patterns with the two comparison mutagenic carcinogens. In the analyses using ToxRead, which selects chemicals similar to the target compound based on molecular descriptors and structural alerts, and which uses modeled as well as experimental data, 16 of the 19 chemicals were predicted to be mutagenic. Several of the chemicals predicted to be mutagens were also predicted to be carcinogens. Our results suggest that alternative toxicity testing methods, such as high-throughput screening assays similar to ToxCast hold great promise for filling data gaps, and facilitating useful predictions for mutagenicity and carcinogenicity of structurally-related chemicals using read-across.

1987 Estrogen Provides Protection against B(a)P-Induced Colon Carcinogenesis

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Colorectal cancer (CRC) is the third most common diagnosed cancer and the third leading cause of cancer-related deaths in the United States. The age-adjusted incidence of colon adenomas and colorectal cancer is higher in men than in women. 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 mechanism of action is not known. Using the Polyposis in Rat Colon (PIRC) animal model, we have showed that male animals develop twice as many polyps in the colon than female animals when treated with the environment toxicant, Benzo(a)pyrene [B(a)P]. In this study, to assess the potential protective role of estrogen against polyp formation in the colon, we treated female PIRC rats, depleted of endogenous hormones by ovariectomy (OVX), with B(a)P and measured polyp development. Here, we show that B(a)P treatment of OVX-female PIRC rats increased when compared to control OVX-female animals. When replacing estrogen through silastic capsules, OVX-female PIRC rats treated with B(a)P showed a significant decrease in total colon polyp formation. These results suggest that estrogen does provide protection against B(a)P-induced colon carcinogenesis. Funded by NIH grants SR25GM059994-3, S5USCA163069-04, and G12MD007586-29.

1989 Nrf2 Induction of Antioxidant Response Increases Bioactivation of the Mutagenic Air Pollutant 3-Nitrobenzanthrone


3-Nitrobenzanthrone (3-NBA) is a potent mutagen and suspected human carcinogen detected in diesel exhaust particulate and ambient air pollution. It requires metabolic activation via nitroreduction to promote DNA adduct formation and tumorigenesis. NAD(P)H:quinone oxidoreductase 1 (NQO1) has been implicated as the major nitroreductase responsible for 3-NBA activation. We investigate the roles of human aldo-keto reductases (AKR1C1-1C3) in 3-NBA reduction and found that AKR1C1-1C3 contribute equally to the nitroreduction of 3-NBA in lung epithelial cell lines (A549 and HBEC3-KT) and combined they represent approximately 50% of the intracellular nitroreductase activity towards 3-NBA. These enzymes are induced by Nrf2 signaling which raises the question whether Nrf2 activation can elicit xenobiotic nuclear receptor NR4A1 (Pax8, Mafk) expression in rhabdomyosarcoma is driven by the targetable nuclear receptor NR4A1, Cancer Research, 77(17):732-741, 2017 [3] Hedrick E, and Safe S. Transforming growth factor β/NR4A1 inducible breast cancer cell migration and epithelial to mesenchymal transition (EMT) is p38α (MAPK14) dependent, Mol. Cell. Biol. 37(18):e00306-17, 2017.

1988 Bis-Indole Derived NR4A1 Antagonist Inhibits TGF-β Induced Rhabdomyosarcoma Cell Invasion

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Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma observed primarily in children and adolescents, and current treatment regimens include surgery, radiation, and chemotherapy with cytotoxic drugs. Although so-called cures for one form of RMS is relatively high, a high percentage (>95%) of RMS patients exhibit serious health effects as adults.1 Studies in this laboratory have demonstrated the nuclear orphan receptor NR4A1 is expressed in RMS and exhibits multiple pro-oncogenic activities associated with cell growth, survival, and invasion and these responses are inhibited after treatment with bis-indole-derived NR4A1 antagonists. In this study, we show for the first time that transforming growth factor (TGF-β) (5ng/ml) enhance migration/invasion of RMS cells (RD and SMS-CT) as determined in a Boyden chamber assay. Treatment of these RMS cells with prototypical NR4A1 antagonist 1,1-bis(3-indolyl)-1-(p-hydroxyphenyl) methane (D(C)-p-PhOH/C-DIM8) alone inhibited invasion of these cells and DIM-C-p-PhOH also significantly inhibited TGF-β induced invasion. We also investigated the effects of various kinase inhibitors (SP600125, LY294002, SB203580, and U0126) on TGF-β-induced RD cell invasion where only SB203580 and U0126 inhibited TGF-β induced invasion. These results are similar, in part with previous study showing the kinase induced phosphorylation of NR4A1 resulting nuclear exit of the receptor which forms a complex to degrade SMAD7. However, in these RMS cells, the subcellular location of NR4A1 in the presence or absence of kinase inhibitors was initially problematic due to antibody variability and the location of the receptor is currently being investigated by extraction methods and immunostaining. References: [1] Hudson MM, Ness KK, Gurney JG, et al., Clinical ascertainment of health outcomes among adults treated for childhood cancer, JAMA, 309:2371-81, 2013 [2] Lacey A, Rodrigues-Hoffman A, and Safe S., PAX3-FOXO1A expression in rhabdomyosarcoma is driven by the targetable nuclear receptor NR4A1, Cancer Research, 77(17):732-741, 2017 [3] Hedrick E, and Safe S. Transforming growth factor β/NR4A1 inducible breast cancer cell migration and epithelial to mesenchymal transition is p38α (MAPK14) dependent, Mol. Cell. Biol. 37(18):e00306-17, 2017.

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1990 Combined Use of Sulforaphane and Zinc Provides a Better Protection against Diabetic Cardiomyopathy Than Either One Alone in Type 1-Diabetic OVE26 Mice

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Diabetic cardiomyopathy (DCM) is the main cause of heart failure in diabetic patients. Oxidative stress is considered to be one of the most important pathophysiological factors. Therefore, treatments targeting oxidative stress may represent suitable strategies for the effective prevention of DCM. Nuclear factor-E2-related factor 2 (Nrf2), a transcription factor responsible to oxidative stress, governs the nuclear and cytoplasmic expression of several antioxidant genes. Metallothionein (MT) is a protein that can bind heavy metal ions such as copper and zinc (Zn). It is also a potent scavenger of free radicals because of its high thiol contents. Overexpression of both Nrf2 and MT was reported to protect from DCM, however, whether up-regulation of both simultaneously would provide a better protection against DCM than either alone remains unclear. Considering that sulforaphane (SFN) and Zn are well-defined activator for Nrf2 and MT, respectively, here we tested our hypothesis that combined use of SFN and Zn to up-regulate Nrf2 function and MT expression might provide better protection against DCM than either SFN or Zn alone. Five-week old OVE26 mice that genetically develop type 1 diabetes (T1D) at 1 week after birth were confirmed with hyperglycemia and then treated with SFN (0.5 mg/kg) and/or Zn (5 mg/kg) for 18 weeks. Twenty-three week old OVE26 mice showed typical DC, reflected by cardiac dysfunction, assessed by echocardiography, and significantly oxidative damage, inflammatory and cardiac dysfunction and pathological features were observed. Furthermore, treatment of OVE26 mice either with SFN or Zn significantly prevented the development of DCM, along with up-regulating Nrf2 function and overexpressing MT protein, respectively. The protection from DCM was better in SFN/Zn-treated OVE26 mice than either SFN- or Zn-treated OVE26 mice. Therefore, combined use of SFN with Zn provides a better protection against DCM than use of either SFN or Zn alone in OVE26 mice.

1991 Application of Imaging-Based Nrf2 Pathway Activation to Support a Read across of Phenolic Compounds

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Biological support for read across approaches is gaining major attention. Damage caused by oxidative stress and soft electrophilic compounds are common during chemical induced liver injury. The correct identification of the liability of a compound to cause such damage is essential for mechanism-based testing and adversity prediction. Various studies proposed similar pharmacological compounds are liable for causing oxidative stress-mediated cytotoxicity. The Nrf2-mediated antioxidant stress response is activated after oxidative and electrophile-derived cell stress. Here we evaluated whether fluorescent protein reporter cell lines for the Nrf2 pathway complemented by TempOSeq targeted RNAseq could support a read across for structural similar phenolic compounds. BAC HepG2 GFP-Nrf2 and GFP-SRXN1 reporter cell lines were exposed to 6-hydroquinone like compounds with redox-cycling potential, 12 redox-cycling and showed an increased PAR association. TFII-I regulates the calcium- dependent gene expression and all compounds that induced SRXN1-GFP also induced stabilization and nuclear translocation of Nrf2-GFP. Cell death was induced at high concentrations for all redox-cycling phenols. Dual-exposure of hydroquinones showed an additive dose effect. The redox-cycling negative phenols were inactive in both Nrf2-GFP activation as well as SRXN1-GFP induction. Alkylated phenols induced onset of cell death, likely independent from oxidative stress induction. To deepen our mechanistic understanding of the phenol-induced toxicity, we also used TempOSeq targeted RNAseq of ~3000 toxicity related genes, including Nrf2 target genes, in HepG2 and cryopreserved primary human hepatocytes. The transcriptomics analysis confirmed the selective activation of Nrf2 target genes by redox-cycling phenols and did provide further insight in the differential pathway activation by the three different phenol groups. In conclusion, our data indicate that our Nrf2 GFP-BAC reporter cell models are a valid test system to provide biological support for read across in cases of anticipated oxidative stress activation. This work was supported through the EU H2020 EU-ToxRisk project (grant agreement 681002).

1992 Caffeic Acid Derivatives Are Effective Bacteriostatic Compounds against Paenibacillus larvae by Increasing Oxidative Stress

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American Foulbrood (AF) is a deadly bacterial disease affecting large numbers of pupal and larval honey bees. AF is caused by the endosporforming bacterium Paenibacillus larvae (PL). Propolis is a product of bee foraging and is a resinous substance derived from botanical substances found primarily in trees, and used in the hive to fill small structural gaps. It has been previously reported that propolis contains a variety of organic compounds that have putative biological activities. A number of compounds found in propolis come from the class of caffeic acid esters and have been shown to have anti-bacterial activity against PL. In this study, six different caffeic acid esters were synthesized and tested for their activity against PL to determine the minimum inhibitory concentrations (MICs). Caffeic acid isopropenyl ester (CAIE), Caffeic acid benzyl ester (CABE), and Caffeic acid phenethyl ester (CAPE) were the most effective in inhibiting PL growth with MICs of 125 μg/ml when used individually, and a MIC of 31.25 μg/ml when used in combination against PL. To determine a possible mechanism of action, flow cytometric analysis and oxidative stress quantifications were utilized to assess the effect of CAIE, CABE, and CAPE on PL cells. Our results indicated all three compounds (CAIE, CABE, and CAPE) inhibited bacterial growth through a bacteriostatic effect, which revealed no lysis of PL cells. Moreover, incubation with CAIE, CABE, and CAPE at or above their given MICs significantly increased oxidative stress within PL after 18 hours. These data indicate that caffeic acid esters are potent bacteriostatic compounds against PL and may inhibit bacterial growth by increasing oxidative stress in PL cells leading to cell cycle arrest. Future research will identify the possible biological targets (DNA, protein or lipid) of these oxidants in order to further elucidate a possible mechanism.

1993 The General Transcription Factor II-I Increase PAR-Association during ROS-Mediated Cell Death

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2,3,5-(Glutathion-S)-hydroquinone (TGHQ) is a nephrotoxic and nephrocarcinogenic metabolite of hydroquinone. TGHQ generates ROS causing hyperactivation of poly(ADP-ribose) polymerase-1 (PARP-1) and reciprocal increases in intracellular calcium concentrations ([iCa2+]2). These data indicate that caffeic acid esters are potent bacteriostatic compounds against PL and may inhibit bacterial growth by increasing oxidative stress in PL cells leading to cell cycle arrest. Future research will identify the possible biological targets (DNA, protein or lipid) of these oxidants in order to further elucidate a possible mechanism.

This work was supported through the EU EU-ToxRisk project (grant agreement 681002).

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1994 Identification and Characterization of Metallothionein-3 as a Pesulfide-Binding Protein

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Persulfides/poly sulfides which contain sulfane sulfur atoms with six valence electrons and no charge (S\(^n\)) exhibit high nucleophilicity and play a role in redox homeostasis. For example, persulfides/poly sulfides can scavenge hydrogen peroxide (H\(_2\)O\(_2\)) and also capture electrophiles such as methylmercury (MeHg), leading to formation of sulfur adducts. We recently reported that cysteinyl-tRNA synthetase 2 produces persulfide-binding protein (PSPB) during protein translation (Akaike T et al. Nature Comm 2017). We also found that there are a variety of PSPBs in hepatic cytosol of mouse and that GSTP1 is identified as a PSPB to capture MeHg, thereby yielding (MeHg)S with less toxicity (Abiko Y et al. Chem Res Toxicol 2015). The purpose of present study is to identify and characterize PSPB from mouse brain, which is susceptible to oxidative stress. Separation of the cytosolic proteins by column chromatography with derivatization of sulfane sulfur atom by addition of [B-(4-hydroxyphenyl)ethyl]iodoacetamide, followed by LC-MS analysis revealed that 16 kDa protein highly purified on SDS-PAGE was identified as metallothionein-3 (MT3). Recombinant human MT3 contained multiple sulfane sulfur atoms in the molecule and exhibited antioxidant capability. Knockdown of MT3 significantly enhanced H\(_2\)O\(_2\) - and MeHg-induced cytotoxicity in human glioblastoma U87 cells. These results suggest that MT3 is a PSPB and the sulfane sulfur atoms in the protein appear to contribute to protection against oxidative and/or electrophilic stresses.

1995 Cholesterol Biosynthesis Dysruptors Alter the Redox Biology of Developing In Vitro Cultures of Primary Rat Cortical Neurons

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The brain is responsible for the synthesis of its own cholesterol because the Blood-Brain Barrier isolates neural pools from systemic and dietary cholesterol. One of the most critical enzymes in the regulation of sterol metabolism is 24-Dehydrocholesterol Reductase (DHCR24). DHCR24 was first shown to be a neuroprotective protein linked to Alzheimer’s Disease but its primary purpose was ultimately determined to be the conversion of desmosterol to cholesterol. In fact, the accumulation of desmosterol caused by mutations in the DHCR24 gene leads to devastating clinical symptoms ranging from craniofacial deformities to psychomotor developmental delay and even premature demise. Based on both clinical and experimental evidence DHCR24 is considered essential for proper neurodevelopment. Our group recently identified a number of US FDA-approved drugs that selectively inhibit DHCR24. Using small molecule inhibitors, our goal is to determine whether these pharmacologically pose an unknown health risk to humans. To determine the impact of pharmacologically inhibiting DHCR24, four well-characterized inhibitors - Imatinib (Gleevec), Pregnenolone (PS), Triparanol (TRP), and U-18666A (U) - were screened in primary rat neuronal cultures for changes in neuronal morphology. Primary cortical neurons grown in vitro for three days were exposed to 100 nM of each compound for 72 hours, a pharmacologically relevant concentration for Gleevec and PS. Cells were probed for various markers of mitochondrial and protein stress using immunocytochemistry including TOM20 (mitochondrial morphology) and FK2 (ubiquitin marker) and quantified for NST (neuron specific tubulin) and DAPI (nuclear morphology). Exposure to TRP and U results in a dramatic reduction in the volume of neuronal processes, increased mitochondrial retraction, and increased ubiquitin levels. Cultures exposed to Gleevec showed modest elevations of ubiquitin levels. PS, an endogenous downstream metabolite of cholesterol biosynthesis, demonstrated none of the aforementioned phenotypes. These data demonstrate that known DHCR24 inhibitors can impede process outgrowth and also concurrently induce mitochondrial and protein stress. These findings hint to an important need to test DHCR24 activity of future drug candidates, especially as it relates to developmental neurotoxicity.

1996 Synergistic Cytotoxic Responses of Co-exposure to Mixtures of Acrolein and Formaldehyde through Oxidative Stress on Human Bronchial Epithelial BEAS-2B Cells


FA (formaldehyde) and ACR (acrolein) are common pollutants in air, and the adverse health effects may not only result from their individual toxicity but also from the combined toxicity. However, combined toxicity of these pollutants has been unknown so far. This study was aimed at investigating the combined cytotoxicity of single FA and ACR in human bronchial epithelial BEAS-2B cells. IC\(_{50}\), of FA (85.49 µM) and ACR (7.63 µM) estimated by CCK-8 assay showed that ACR is more toxic than FA. Significant dose-dependency and time-course could be observed on the cytotoxic effects based on CCK-8 assay, when BEAS-2B cells were exposed to subcytotoxic concentrations (5, 10, 20, 40 and 80 µM) of FA and subcytotoxic concentrations (0.5, 1, 2, 4, 6 and 7.5 µM) of ACR alone or in combinations. Significant synergistic effects (<0.05) were noted upon exposure to 10 (20,40 and 80) µM of FA + 7.5 µM ACR. Moreover, ROS levels, GSH levels and the activities of antioxidants (CAT, SOD and GPX) significantly increased (<0.05) in the synergistic style, when Beas-2B cells were exposed to 40 (80) µM of FA + 7.5 µM of ACR. In addition, the cell viabilities of Beas-2B cells at 40 (80) µM of FA + 7.5 µM of ACR were significantly elevated from 88% (57%) of control to 111% (100%) of control by antioxidants NAC, and the synergistic effects turned to additive effects. So oxidative stress is an important mechanism responsible for the synergistic cytotoxic responses of co-exposure to mixtures of FA and ACR. Taken together, prolonged exposure to mixtures of FA and ACR at subcytotoxic concentrations could induce significantly synergistic effects on cytotoxicity of BEAS-2B cells. And the oxidative stress not only can be responsible for the single toxic effects of FA and ACR but also for the combined toxicity.

1997 Inhibiting 7-Dehydrocholesterol Reductase Elevates Protein Adduction and Depletes Intracellular Glutathione

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The brain is vulnerable to oxidative injury in part due to its high lipid content. In fact, more than a quarter of the body’s cholesterol (Chol) resides in the brain. Although Chol is unreactive, its immediate precursor 7-dehydrocholesterol (7DHC) is one of the most oxidizable lipids in biology. Under most conditions neurons have low levels of 7DHC. However, inhibiting DHCR7, the enzyme responsible for converting 7DHC to Chol, causes an increase in 7DHC levels. We have identified over 100 US FDA-approved drugs that inhibit DHCR7. Despite evidence that chronic elevations of 7DHC is neurotoxic, no mechanism of toxicity is established for acute exposure to a DHCR7 inhibitor. Protein adduction by reactive oxysterols derived from 7-DHC is a likely key event in DHCR7 inhibitor toxicity. To study sterol-protein adduction events our lab developed traceable sterol compounds that contain alkyne groups such as alkynyl-7DHC (aDHC) and a reactive oxysterol, alkyne-7DHC epoxide (aDHcep). The alkyne moiety offers the means to analyze adduction with immunoblots or proteomics using “click” chemistry. Using these tools in cells deficient in DHCR7 we identified a number of adducted adaptive response proteins such as glutathione reductase. Utilizing the neuroblastoma cell line, Neuro2a, and the well-characterized DHCR7 inhibitor, AY-9944 (AY), we tested whether elevated levels of 7DHC impair the cellular adaptive response. Exposing Neuro2a cells to AY for 48h decreased total glutathione levels in a concentration-dependent manner down to 20% of control. We next incubated cells with a traceable sterol precursor that is biosynthetically converted to aDHC for 48h concurrently with 100 nM AY. Immuno blot analysis revealed AY treatment increased protein adduction 2.5±0.5-fold. To test for an interaction between glutathione and reactive oxysterols we depleted intracellular glutathione levels with a 500 uM BSO treatment for 24h followed by a 4h exposure to a7DHcep. a7DHcep alone increased protein adduction with a 10 μM, 4h treatment elevating adduction 3±0.4-fold. However, pretreatment with BSO profoundly reduced a7DHcep-induced adduction at all concentrations tested. Our data suggest that chemical inhibition of DHCR7 increases protein adduction and that targeting glutathione levels provides a means to offset 7DHC-induced proteotoxicity. Supported by NIEHS T32 ES00267 (PW) ES024133 (NP).
Current consensus is that reactive oxygen species (ROS) produced by environmental chemical exposure potentially mediate redox signaling through S-oxidation of thiols with lower pKa value on sensor proteins (e.g., phosphatases), associated with substantial activation of their effector molecules (e.g., kinases, and transcription factors). We found that 9,10-phenanthraquinone (9,10-PQ) contaminated as an abundant polycyclic aromatic hydrocarbon quinone in diesel exhaust particles and particulate matter 2.5, produces excess ROS through redox cycling by interacting with electron donors in cells (Kumagai Y et al. Chem Res Toxicol 2002; Taguchi K et al. Free Radic Biol Med 2007 and 2008), indicating that 9,10-PQ might modulate redox signaling through ROS generation. In this study, we examined that 9,10-PQ could activate epidermal growth factor receptor (EGFR) signaling in A431 cells through S-oxidation of its negative regulator protein tyrosine phosphatases (PTPs) such as PTP1B, and thus affect cell survival. Exposure of A431 cells to 9,10-PQ activated EGFR-ERK1/2 signaling, coupled with the decrease of cellular PTPs activity. ROS produced during 9,10-PQ exposure were abolished by pre-treatments with 3-phosphoglycerate (3PG) scavenger. Under this condition, PEG-CAT significantly blocked the 9,10-PQ-mediated activation of EGFR-ERK1/2 signaling, indicating involvement of 9,10-PQ-mediated ROS generation in this activation. Consistent with this, a CAT protected diminished recombinant HPTP1B activity caused by 9,10-PQ. UPLC-MS analysis revealed that a 9,10-PQ oxidized PTP1B was present. Interestingly, an EGFR inhibitor enhanced cytotoxicity of 9,10-PQ, indicating that EGFR-ERK1/2 activation is a cytoprotective response to the toxicity. Collectively, these results suggest that 9,10-PQ-activated ROS activate EGFR-ERK1/2 signaling presumably through oxidative modification of PTP1B and, at least in part, the activation of the signal cascade plays a role in cellular defense against 9,10-PQ in A431 cells.

1999 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)-Elicited Metabolic Reprogramming in Primary Mouse Hepatocytes Supports Antioxidant Defense Mechanisms

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Induction of the M2 isofrom of pyruvate kinase (PKM2) is a central feature of the Warburg Effect in cancer cells. The reduced glycolytic flux causes accumulating upstream intermediates to be redirected to antioxidant and/or proliferation pathways. We have previously shown that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) dose-dependently induces PKM2 in normal differentiated C57BL/6 mouse hepatocytes. We hypothesize that PKM2 induction supports defensive responses to ary hydrogen carbons (AHR)-mediated oxidative stress. To investigate the role of TCDD-elicited PKM2 induction in central carbon metabolic reprogramming, tracer studies were performed using primary hepatocytes from PKM2 isoform-specific conditional knockout (PKM2-fl) and wild-type (WT) mice. The hepatic arterial versus portal vein (HA:PV) ratio of [13C]-labeled Ru5P, -3-phosphoglycerate (3PG), -glycerine, -serine, -5-methyltetrahydrofolate, -pyruvate, and -lactate, suggesting redirection of glycolytic intermediates toward NADPH-producing pathways. In PKM2-fl mice, earlier increases in 3PG is suggestive of increased glycolytic flux, while reduced levels of 13C-labeled Ru5P, serine, glutathione, and TCA cycle intermediates indicate central carbon metabolites were not redirected as in TCDD-treated PKM2-fl hepatocytes. Moreover, dose-dependent treatment with H2O2 showed increased sensitivity to oxidative stress in PKM2-fl hepatocytes compared to PKM2-fl controls following TCDD treatment, demonstrating its crucial role in antioxidant defenses. Collectively, these data support the hypothesis that TCDD-elicited PKM2 induction is a novel antioxidant defense response that combats oxidative stress and increases cell survival.
2002 Manganese Superoxide Dismutase (MnSOD) Attenuates Hyperoxia-Induced Cell Death and Alters ERK Activation

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Supraphysiological concentrations of oxygen (hyperoxia) are often utilized to treat patients with respiratory failure. However, prolonged exposure to hyperoxia results in severe lung injury and pulmonary cell death. Hyperoxia generates reactive oxygen species (ROS) in the mitochondria, damaging their structure and function. Here, we demonstrate that lung epithelial cells overexpressing MnSOD (+200%) are more resistant to oxidative injury, and that the activation of ERK plays a role in such protection. To test whether the overexpression of MnSOD in mitochondria protects cells from hyperoxia-induced cell injury and alters ERK signaling, fibroblasts overexpressing MnSOD or catalase in mitochondria were exposed to 95% oxygen for up to 6 days, mimicking hyperoxic conditions. Mitochondrial injury and cell death were assessed by MTT, nuclear condensation, and Trypan Blue exclusion assays. Most (>94±0.8%) cells overexpressing MnSOD (2.5-8 fold) in mitochondria survived after exposure to hyperoxia for 4 days compared to vector controls. This protective effect by mitochondrial MnSOD was associated with increased DCF labeling. Overexpression of catalase offers protection against hyperoxia. Higher basal levels of ERK activation, determined by in vitro kinase assays, were observed in MnSOD overexpressers in hyperoxia. There was a correlation found between ERK activation and survival in hyperoxia. Pretreatment with the MEK inhibitor, PD98059, reduced ERK activity and cell death in hyperoxia. The data suggest that moderate overexpression of MnSOD in the mitochondrial significantly protects against hyperoxic non-apoptotic cell death. Intracellular H2O2 may play an important role in mediating ERK activation and modulating survival in response to hyperoxia.

2003 Redox Proteomics Analysis Reveals Slc7a11 Restores Age-Dependent Change of Redox State of Proteins in Pathways of Protein Turnover and Cell Death

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System Xc− is an antiporter that imports cystine (CySS) and exports glutamate. It plays a critical role in cell defense against oxidative stress because cysteine (Cys) reduced from CySS is used for and limits synthesis of GSH. Specificity of system Xc− is conferred by the Slc7a11 subunit. Down-regulation of Slc7a11 is responsible for oxidation of extracellular Cys/CySS redox potential (EC50/CySS) in lung fibroblasts from old mice. However, whether expression of Slc7a11 affects intracellular redox environment of these mouse lung fibroblasts remains unexplored. The purpose of this study is to evaluate the effect of Slc7a11 on the redox state of intracellular proteins. Redox proteomics was used to determine intracellular protein oxidation. IodoTMTs were used to label and reduced oxidized Cys residues in primary lung fibroblasts from young and old mice, as well as old fibroblasts transfected with Slc7a11. After labeling, 3 kinds of fibroblasts were mixed, digested, and affinity purified prior to multiplexed MS/MS. The ratio of oxidized/reduced forms (i.e., redox state) of proteins identified in each kind of fibroblast was determined. Change of ratio between different kinds of fibroblasts was calculated. GO and IPA were conducted for protein function and pathway enrichment analyses. STRING and Cytoscape were used for protein-protein interaction analysis. Slc7a11 over-expression restored global age-dependent trend of oxidation of cysteine residues in multiple peptides. Specifically, redox states of 151 proteins were changed in old fibroblasts compared to young fibroblasts. Slc7a11 over-expression restored redox states of 104 of these proteins. GO analysis based on those 104 proteins showed cell adhesion, protein translation and RNA binding were the top enriched functions. IPA exhibited age-dependent changes of redox states of components in pathways of protein translation initiation, ubiquitin-proteasome-mediated degradation and cystoskeleton-associated signaling were reversed by Slc7a11 over-expression. Cell death and survival were predicted to be the most affected with necrosis being on the top. This study finds that aging results in changes of redox states of multiple proteins, including those involved in protein turnover and cell survival, and that targeting Slc7a11 can restore the redox states of these proteins.

2004 The Activation of NF-κB in Airway Epithelia Protects against Hyperoxia-Induced Proinflamatory Lung Injury

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The therapeutic use of mechanical ventilation with hyperoxia, often used for hypoxic conditions, can cause oxygen toxicity and inflammatory acute lung injury (ALI). ALI induced by hyperoxia can lead to excessive proinflammatory responses, endothelial and epithelial cell damage, and alveolar edema. It has been demonstrated that NF-κB activation has a protective effect on cultured lung epithelial cells, making them less susceptible to oxidative cell injury. The objective of this study is to investigate the role of NF-κB activation in airway epithelial cells in a mouse model of hyperoxia-induced lung injury. CC101-i-kbαSR transgenic mice, that do not express the wild-type gene that activates NF-κB, were exposed to 95% oxygen for 3 days. The bronchoalveolar lavage fluids (BALF) and lung tissues of the mice were collected and examined. Transgenic mice showed significantly more severe lung injury than wild-type mice. The more severe lung injury was characterized by higher wet/dry weight ratios of lung tissue and increased total protein content in BALF. Elevated neutrophil infiltration and extracellular HMGB1 accumulation in the BALF were associated with the more severe injury in transgenic mice. In addition, we found that prolonged exposure of cultured lung epithelial cells to hyperoxia induced the release of HMGB1. To determine if deficient NF-κB activation also leads to higher levels of HMGB1 in hyperoxia, human bronchial epithelial (HBE) cells were exposed to BAY 11-7082, an inhibitor of NF-κB activation. Higher levels of extracellular HMGB1 were indeed observed in those cells cultured with BAY 11-7082. These results indicate that the NF-κB signaling pathway in airway epithelial cells plays an important protective role against HMGB1-mediated hyperoxic inflammatory lung injury.

2005 Macrophages Hyperoxia-Compromised Phagocytic Function Improved by Nrf-2 Inducer

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Prolonged exposure to hyperoxia can compromise the ability of alveolar macrophages to phagocytose invading pathogens due to excessive production of intracellular reactive oxygen species (ROS). This can lead to the increased susceptibility to pulmonary infections observed in ventilated patients. Induction of the nuclear factor 2-erythroid-related factor (Nrf-2) pathway has been shown to regulate intracellular levels of ROS. The present study investigates whether resveratrol (RES), a Nrf-2 inducer, can ameliorate hyperoxia-compromised macrophage function. RAW 264.7 cells, a murine macrophage-like cell line, and bone marrow-derived macrophages (BMMDM) were exposed to hyperoxia for 24 hours in the presence or absence of RES. Macrophages treated with RES had significantly higher phagocytic activities compared to the control macrophages treated with vehicle alone. In addition, hyperoxia-induced accumulation of extracellular HMGB1, which can also impair macrophage functions, was reduced in RES treated macrophage culture media. These observations are accompanied with the increased levels of endogenous antioxidant compound, HO-1, a down-stream event of Nrf-2 activation. These data suggest that pharmacological activation of Nrf-2 pathway is efficacious in attenuating hyperoxic adverse effects on macrophages. Thus RES may provide a therapeutic approach to improve innate immunity of patients on ventilator in the treatment of ventilator associated pneumonia.

2006 Supplementation with Omega-3 Fatty Acids Potentiates Oxidative Stress in Human Airway Epithelial Cells Exposed to Ozone

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The health benefits of dietary fish oil consumption have been widely reported. Eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, two omega-3 fatty acids found in fish oil, have been associated with anti-inflammatory and pro-resolving effects through their displacement of arachidonic acid (AA) and metabolism to specialized pro-resolving mediators (SPMs). However, the 5 and 12 double bonds in EPA and DHA reduce their potential to be resolved by peroxidation by O2., a major photochemical pollutant to which millions of Americans are exposed. Products of unsaturated fatty acid peroxidation by O2. have been proposed to mediate some of the functional and inflammatory effects of O2. exposure. Thus, cellular supplementation with omega-3 fatty acids has the potential to both support the inflammatory response induced by O2.
inhalation as well as promote its resolution. To investigate this paradox, we supplemented 16-HBE human airway epithelial cells (HAE) expressing the fluorogenic glutathione redox potential (E_GSH) sensor roGFP overnight with EPA, DHA, the monounsaturated fatty acid oleic acid (OA), or the saturated fatty acid stearic acid (SA). Alterations in E_GSH were monitored in real time using live-cell microscopy during exposure to OA. We found that supplementation with EPA and DHA, but not OA or SA, caused a marked potentiation of the O_2•^- induced increases in E_GSH evident as both an accelerated response time and increase in the magnitude of response. Notably, the potentiation of GSH oxidation occurred at O_2•^- concentrations approximating the current National Ambient Air Quality Standard level. However, supplementation with DHA or OA did not significantly alter the production of the pro-inflammatory mediator prostaglandin E_2 (PGE_2) induced by O_2•^- exposure. These results suggest that membrane fatty acid saturation is a determinant of the oxidative effects, but does not modulate the production of pro-inflammatory eicosanoids in HAE exposed to O_2•^- These findings may have implications for dietary recommendations for populations exposed to O_2•^- This abstract does not necessarily reflect US EPA policy.

2007 Ambient Air PMx Reactive Oxygen Species Generation Capacity
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Differences in PM toxicity based on particle source and composition is still a matter of scientific debate that has significant regulatory repercussions. Although the World Health Organization uses similar concentration-response functions for PM emitted from all combustion sources (equitoxicity), there is an increasing body of evidence highlighting the differences in PM toxicity. In order to identify the differences in PM toxicity, the oxidative potential of particular matter PM has been evaluated in the frame of large European research projects (TRANSFORM, HEALS, CROME, ICARUS). The analysis was based on PM chemical speciation data over a period of 6 years (2012–2016) distinguishing between sampling sites typical of traffic and urban background pollution. The oxidative potential of PM was evaluated using the dichrothion (DHTT) assay. Based on the measurements and chemical analysis data used for source apportionment, it was found that during winter time, all PM fraction levels were higher at the urban background sites than at the traffic sites, as a result of the extensive biomass-fueled domestic space heating in several European cities. Regarding the PM oxidative potential, it was found that their toxicity is higher and is driven by the presence of organic compounds (PAHs and phenols) and inorganic elements (toxic metals) present in the lower PM fractions (PM1 and below), as a result of the process of formation of the smaller particles (accumulation mode) that results in higher concentrations (per mass of PM unit) of these oxidative components. The contribution of different sources to the oxidative properties of various PM fractions, was determined using Positive Matrix Factorization analysis. At the urban background sites, biomass burning contributed to 63% of the DHTT activity of PM, while vehicular emission and oil combustion together accounted for 35%. On the contrary, at the traffic sites, contributions contributed to 31% to the DHTT activity of PM, while secondary oxidation processes contribution was up to 57%. Overall, these results highlight that biomass burning results not only in higher PM levels, but also to PM that are characterized by a higher capacity of oxygen species generation.

2008 Critical Windows of Redox Modulation for Pancreas Development and Embryo Survival

Altered development of the pancreas by toxicant exposure can lead to altered pancreas morphology that has been linked to later-life diabetes. In this study, zebrafish (Danio rerio) was used as a model to compare critical windows of exposure to redox modulators for pancreas organogenesis and embryonic survival. Transgenic embryos were used to evaluate endocrine (ins:GFP and gcga:GFP) and exocrine (ptfaa:GFP) pancreas development. Embryos were exposed to water and dimethyl sulfoxide (DMSO) as controls, antioxidants N-acetyl cysteine (NAC) and sulforaphane (SFN), and pro-oxidants tert-butyl hydroperoxide (tBOOH) and tert-butyl-hydroquinone (tBHQ) at 24, 48, or 72 hours post fertilization (hpf) to assess endocrine islet and exocrine pancreas morphology at 96 hpf. tBOOH significantly decreased exocrine pancreas length at 48 and 72 hpf by 15% and 7% respectively. Both chemical-, stage-, and cell-type specific effects of redox modulation were observed on the endocrine pancreas. Endocrine pancreas was most sensitive at 48 hpf, where tBHQ resulted in β-cell cluster area reduction by 22.83% and 26.45% respectively, and increased variance islet frequencies by 30.3% and 36.67% respectively. On the other hand, antioxidants NAC and SFN significantly increased β-cell cluster area by 20.68% and 25.7% respectively. Cluster area of a-cells was only affected at 48 hpf where tBOOH and tBHQ decreased a-cell cluster area by 8.87% and 7.9% respectively. In addition, dose response curves were generated for both pro-oxidants to identify critical window of exposure and LC_50. In contrast to pancreatic endpoints, embryos were most sensitive to pro-oxidants at 72 hpf. Estimated LC_50 values at 24, 48 and 72 hpf for tBOOH were 6.90, 1.56 and 0.57 μM respectively, and for tBHQ, (HAAs) (H2O2) and acetamides. Cytotoxicity-assays revealed a structure-activity relationship wherein iodoacetic acids and acetamides are the most toxic congeners, followed by bromo- then chloro-DBPs. Unexpectedly, NF2-mediated oxidative stress was strongly induced by iodo- and bromoacetamides but not by HAAs, highlighting the differences in toxic pathways. The objective is to investigate their exact mechanisms via chemical proteomics by employing an alkyne-modified iodoacetamide chemical probe for in-gel fluorescence imaging. Accordant with cytotoxicities, iodo- and bromo-DBPs bind to more proteins than chloro-DBPs, as evidenced by increased probe competition. Interestingly, halogenated acetic acids and acetamides were found to bind to different proteins although both of them have been reported to be cysteine reactive. We further identified the target proteins using biotin-mediated affinity pulldown mass spectrometry analysis, which revealed that more than 500 proteins are modified by these DBPs. The identified proteins were compared to those of essential genes, previously determined by CRISSP, to identify the major cytotoxic pathways. Consistent with in-gel fluorescence imaging, the protein targets of halogenated AAs and acetamides were different. For example, phosphoglycerate kinase (PGK1) was identified as a specific protein target of iodoacetamide. Of the modified proteins, a recurring theme was the covalent binding of anti-oxidant enzymes such as peroxiredoxins. We validated our proteomics results using enzyme kinetic assays of anti-oxidant enzymes. Thus, we have provided the first evidence that cysteine reactive halogenated DBPs may induce diverse toxicities by targeting different proteins. Application of chemical proteomics to investigate the toxic mechanisms of broader classes of DBPs is of great interest.

2009 Chemical Proteomics Reveals Different Toxic Pathways of Halogenated Acetic Acids and Acetamides

Epidemiological and animal exposure studies alike both highlight the potential adverse health effects of disinfection-by-products (DBPs), but the toxic mechanisms of DBPs remains elusive. Our previous studies profiling pre- and post-chlorinated drinking water samples have demonstrated that NF2-mediated oxidative stress is the primary toxic pathway of DBPs. Motivated by this, we chose to investigate the toxic pathways (i.e. protein targets) of two prominent classes of DBPs: monohalogenated acetic acids and acetamides. Cytotoxicity-assays revealed a structure-activity relationship wherein iodoacetic acids and acetamides are the most toxic congeners, followed by bromo- then chloro-DBPs. Unexpectedly, NF2-mediated oxidative stress was strongly induced by iodo- and bromoacetamides but not by HAAs, highlighting the differences in toxic pathways. The objective is to investigate their exact mechanisms via chemical proteomics by employing an alkyne-modified iodoacetamide chemical probe for in-gel fluorescence imaging. Accordant with cytotoxicities, iodo- and bromo-DBPs bind to more proteins than chloro-DBPs, as evidenced by increased probe competition. Interestingly, halogenated acetamides and HAAs were found to bind to different proteins although both of them have been reported to be cysteine reactive. We further identified the target proteins using biotin-mediated affinity pulldown mass spectrometry analysis, which revealed that more than 500 proteins are modified by these DBPs. The identified proteins were compared to those of essential genes, previously determined by CRISSP, to identify the major cytotoxic pathways. Consistent with in-gel fluorescence imaging, the protein targets of halogenated AAs and acetamides were different. For example, phosphoglycerate kinase (PGK1) was identified as a specific protein target of iodoacetamide. Of the modified proteins, a recurring theme was the covalent binding of anti-oxidant enzymes such as peroxiredoxins. We validated our proteomics results using enzyme kinetic assays of anti-oxidant enzymes. Thus, we have provided the first evidence that cysteine reactive halogenated DBPs may induce diverse toxicities by targeting different proteins. Application of chemical proteomics to investigate the toxic mechanisms of broader classes of DBPs is of great interest.

2010 Possible Prooxidant Actions of Tetra bromobisphenol A
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Tetra bromobisphenol A (TBBPA) and its derivatives are used as flame retardant in consumer products such as circuit boards and household electrical equipment. Recently, US EPA rejected a petition to mandate manufacture to conduct direct testing on TBBPA. Emerging evidences suggest that TBBPA exposure in zebrafish embryos and fish Channa punctatus (Sharma 2018 Drug and Chemical Toxicology, 1-6) increased oxidative stress and caused developmental toxicity. We hypothesize that the prooxidant actions of TBBPA is responsible for the increased oxidative stress, which may in turn cause damage to DNA, protein and lipids. To understand the TBBPA-induced alterations in cellular redox status, we have investigated the prooxidant effects of TBBPA in comparison with four other phenolic compounds, viz., phenol (P), 4-bromophenol (BP), 2,2,6,6-Tetramethylpiperidin (TBBPA), and 4-nitrophenol (NP). The phenolate radicals of P, BP, TBBPA, and NP, generated in situ via 1-electron-oxidation of the corresponding phenol (0.1 mM each) in the HRP/H2O2 system, were allowed to react with equal amount of rifampicin (0.1 mM) and monitored at 472 nm. In the rifampicin assay, higher rates of oxidation were observed with the radicals of BP (50.71 ± 4.75 μM/min) and P (36.24 ± 6.65 μM/min) as compared to those of TBBPA (23.38 ± 18.41 μM/min) and NP (13.2 ± 3.81 μM/min) and the corresponding control assay (17.33 ± 6.25 μM/min).
2012 Mucin Muc5ac Deficiency Enhanced Airway Susceptibility to Environmental Toxins

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Gel-forming mucins protect respiratory tracts. MUC5AC is the major secreted airway mucin that is associated with various diseases including asthma, chronic obstructive pulmonary disease, and fibrosis. To determine the role of Muc5ac in airway disorders in which abnormally heightened mucus is featured, Muc5ac−/− mice and wild type mice (Muc5ac+/+) were infected with respiratory syncytial virus (RSV, intranasal infection) or exposed to ozone (0.3 parts per million). Pulmonary and nasal airway injuries were assessed by bronchoalveolar lavage and histopathologic analyses. RSV was detected more intensely in nasal (septal epithelium and sub-epithelium, blood vessels) and pulmonary (bronchial epithelium, pleura) airways of treated, but not wild type mice. Impaired nasal and pulmonary airway defenses against virus and ozone. Subepithelial mucous glands in nasal airways may contribute to compensatory protective mechanism of Muc5ac−/− mice.

2013 Effect of Diet and Occupational Exposure in Different Rat Strains on Serum Biomarkers and Peripheral Blood Mononuclear Cell Telomere Length: Development of an Animal Model to Examine the Exposome


The exposome is a measure of all exposures of an individual and how those exposures relate to health. Important components of the exposome include lifestyle (diet), environmental and occupational exposures, and individual genetic predisposition. Mapping of the exposome will improve the understanding of disease and aid in prevention strategies and possible cures of many diseases. The goal was to develop an experimental model of the exposome by collecting biological samples during critical life stages of an exposed animal that are applicable to worker populations. Genetic contributions were assessed using three strains of male rats with different genetic backgrounds. A. Martinez, S. Kleeberger, and an unidentified, soil sample-based bacteria) would grow significantly faster in the presence of 3-NBA. Nitro-reductases secreted from the bacteria metabolized 3-NBA, and a current investigation of a NCBI gene database for these bacteria will attempt to locate which genes allow the bacteria to produce these nitro-reductases. There are strong matches with genes such as CB03577 and CB00878. 3-NBA was injected into a solution of nutrient broth with bacteria and left to grow for two hours. After the bacteria were allowed to grow in this solution, the solution would turn pink (from yellow), and its wavelength absorbance would show peaks resembling those of 3-Aminobenzanthrone. 3-ABA is significantly less toxic to 3-NBA and its metabolism is extraordinarily harmful and leads to DNA adducts. Allowing the bacteria to metabolize 3-NBA, rather than allowing the human body to do
Occupational and indoor exposure to asbestos can lead to the development of pulmonary fibrosis years after exposure has ceased, leading to significant morbidity and mortality. Asbestos fibers can lodge within the bronchoalveolar duct junctions and small airways of humans and mice respectively, persisting for years. Although multiple cell types have been implicated as important participants in the development and progression of asbestos-induced lung fibrosis, the specific mechanisms and key cellular players involved are not known. Using a comprehensive combination of unbiased single cell transcriptional profiling (scRNA-Seq), genetic lineage-tracing, flow cytometry and in situ RNA hybridization, we tested the hypothesis that monocyte-derived alveolar macrophages are key drivers of asbestos-induced pulmonary fibrosis via epithelial cell injury and fibroblast proliferation. C57Bl6 mice were exposed to TiO$_2$ (control) or asbestos fibers intratracheally. Lungs were harvested 14 days later to capture the early stages of pulmonary fibrosis and scRNA-Seq libraries were prepared from cell suspensions using the 10X Chromium platform. Profiling 24,060 cells identified 24 known cellular populations represented in all experimental conditions. All populations exhibited transcriptional changes during the development of fibrosis. Importantly, the emergence of a new distinct subpopulation of alveolar macrophages was observed in asbestos-exposed animals. This subpopulation was characterized by an immature phenotype and elevated expression of genes known to be causally associated with fibrosis such as Mmp12, Retnla, Chia1 and Pdgfa (involved in fibroblast proliferation). Furthermore, these cells expressed Itgam and Cxcr2, suggesting a monocyte origin. Remarkably, this new subpopulation was represented only by cells from asbestos-exposed mice and was absent in control conditions. Flow cytometry, lineage-tracing analyses and immunohistochemistry confirmed this subpopulation to be monocyte-derived alveolar macrophages. Immunofluorescent microscopy confirmed that Pdgfra-expressing cells were specifically recruited to the areas of fibrosis and were located in the proximity of Pdgfra-expressing fibroblasts. Creflox-mediated genetic deletion of this population by targeting Casp8 prevented the development of pulmonary fibrosis. Collectively, these studies are the first to show a causal association between asbestos-induced epithelial lung injury, localized recruitment of monocyte-derived alveolar macrophages and subsequent development of spatially restricted lung fibrosis.

W. McKinney

2016 Lack of Lung Tumor Promotion after Inhalation of a Copper-Nickel Welding Fume in A/J Mice

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The International Agency for Research on Cancer classified welding fumes as genotoxic (carcinogenic to humans) in 2017. The process of stainless-steel welding creates fumes rich in carcinogenic metals such as chromium (Cr). Our lab has previously demonstrated that stainless steel welding fumes promote lung tumors in susceptible A/J mice. Consumables devoid of Cr are being produced and an attempt to limit worker exposures to potentially carcinogenic metals. The aim of this study was to characterize a new copper-nickel (Cu-Ni) fume and then investigate if inhalation of this fume would promote lung tumors in mice using a two-stage (initiation-promotion) model. To determine particle mass size distribution, a Micro-Orifice Uniform Deposit Impactor (MOUDI, model 110; MSP corp., Shoreview, Minn.) with additional Nano-MOUDI stages (MSP model 115) was used. Characterization of the fume indicated that most of the particles were between 0.1 and 1 µm in diameter, with a mass median aerodynamic diameter of 0.43 µm. Male A/J mice (4 - 5 weeks old) were initiated with 3-methylcholanthrene (MCA; 10 µg/g IP) and corn oil and, beginning 1 week later, were exposed to air or Cu-Ni welding fumes for 4 hours/day, 4 days/week, for 9 weeks. At 30 weeks, mice were sacrificed and lung tumor multiplicity and incidence were evaluated. MCA/Cu-Ni welding fume exposure significantly decreased tumor number and tumor size compared to MCA/air controls (7.11 ± 0.93 tumors vs. 15.57 ± 0.75 tumors and 0.57 ± 0.01 mm in diameter vs. 1.15 ± 0.02 mm in diameter, respectively). Future studies are planned to investigate the pneumotoxicity of Cu-Ni fume in A/J mice.

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2017 Functional Significance of the SLC26A4 Gene in Silica-Induced Pulmonary Toxicity


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Occupational exposure to silica may result in potentially fatal diseases such as silicosis and cancer. Understanding molecular mechanisms responsible for silica-induced pulmonary toxicity is of great importance in preventing silicosis and other effects associated with occupational silica exposure. Previous studies in our laboratory identified a correlation between silica-induced pulmonary toxicity and SLC26A4 gene overexpression in the lungs of rats. However, the functional significance of this gene in silica induced pulmonary toxicity is not understood. To determine the role of the SLC26A4 gene in silica-induced pulmonary toxicity, SLC26A4 wild type (WT) and knockout (KO) mice were employed. All mice were exposed to either air or crystalline silica (15 mg/m$^3$, 6 hours/day, 4 days) and pulmonary toxicity was assessed at 1 day, 3 months, 6 months, and 9 months post-exposure. Pulmonary response parameters including, lactate dehydrogenase (LDH) activity, oxidant production, cell counts (including infiltrating neutrophils and alveolar macrophages), and gene expression were monitored. Silica inhalation caused a significant increase in pulmonary toxicity and inflammation in both the WT and KO mouse strains, compared to corresponding air exposed controls. However, there were significant differences (p<0.05) in the measured pulmonary toxicity parameters between silica exposed WT and KO groups. For example, induction of pulmonary inflammation in the silica exposed WT mice was accompanied by a significant increase in infiltration of neutrophils in the lung. This infiltration was vastly different between the WT and KO groups. Specifically, at 3 months post-exposure neutrophil infiltration in the WT mice was 480 fold higher compared to air exposed controls while being 205 fold higher in the KO mice. At 6 months post-exposure, neutrophil infiltration in the WT mice was 192 times higher than air controls while the KO mice had, a significantly lower, 54 fold increase in PMN number, compared to air controls. At 9 months post-exposure neutrophil number was 45 fold higher in WT mice and only 9 fold higher in KO mice compared to air controls. In conclusion, both the WT and KO mice presented with an enhanced inflammatory response with a distinct pattern. However, the severity of silica induced pulmonary toxicity was more in the WT mice compared to the KO mice. These findings support the hypothesis that the SLC26A4 gene does, in fact, play a role in silica induced pulmonary toxicity.

2018 Understanding the Lung-Gut Axis by Modeling the Influence of Welding Fume Inhalation Exposure and Lifestyle on the Profile of Gut Microbiome and Systemic Immune Cells


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The gut microbiome has a regulatory influence on various systemic organs, and altered microbiome diversity correlates with various diseases and pathological conditions. The goal of the current work was to profile and correlate the influence of occupational pulmonary exposure (welding fume), lifestyle (high fat diet) and age on the gut microbiome and immune cell phenotype populations in blood, lung lymph nodes and spleen. Male Sprague-Dawley rats were maintained on a regular chow (RO) or high fat (HF) diet for 24 wk. At wk 7, groups of rats maintained on each diet were exposed by inhalation to stainless steel welding fume (WF: 20 mg/m$^3$ x 3 hr/day x 4 wk x 5 wk) or filtered air until wk 12, at which time some animals from each group were euthanized. A separate set of rats from each group were allowed to recover from WF exposure until wk 24. At these time points, immune cells from various systemic locations were profiled using flow cytometry. The DNA from the lower gut feces was extracted and sequenced for 16s. The ratio of firmicutes to bacteroidetes consistently decreased in RG-fed rats and increased for HF-fed rats over the 24 wk period. This was further exacerbated in WF-exposed animals. Random Forest analysis was employed to identify specific alterations at genus and species level for the various treatments. There was no change in total leukocyte number but there was a significant increase in neutrophils recovered from the blood of rats fed the HF vs the RG diet. In the lungs, there was no change in the leucocyte profile between rats fed WF exposure diets after 7 weeks exposure. However, after a recovery period, lung neutrophil and lymphocyte numbers, in addition to percent of pulmonary macrophages, remained significantly elevated in rats maintained on
the HF diet. In the spleen and lymph nodes, like the lung, WF exposure did not change the response with various diets. However, as the animals aged, the HF diet caused a significantly elevated B'T lymphocyte ratio in both the spleen and mesenteric lymph nodes compared to the RG diet. The percent of CD8+-T-lymphocytes remained elevated in the lymph nodes of the HF but not RG-fed rats. Taken together, the data suggests that diet by itself causes a dysbiosis of normal microbiome and immune populations. This effect is irrevocably exacerbated over time when exposed to a secondary pulmonary insult, such as welding fume.

2019 Reducing the Respiration Rate and Improving Animal Welfare in Inhalation Large Animal Studies by Using a 3D Printer and Designing Specific Masks

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Inhalation large animal dosing requires the use of a facemask to deliver the aerosol to in vivo species. Exposure dosing exposure period can be up to 240 min depending on the target dose. However, the historical masks that have been used by the industry are modified from existing off the shelf clinical or veterinary masks and generic in size and as such are a compromise for their application. Envigo designed and manufactured new masks to improve animal welfare by making them specific for inhalation dosing masks by using 3D printing. Casts were made of various sizes of large animals and photographed at 30° divisions using a 3D-scanner. The individual photos from each cast were then merged into a single 3D image by the AutoCAD/CAM software. Prototype masks and seals were then produced using 3D printer software that could be adhered to the animals face with the use of Velcro straps. Validation consisted of delivering 5% CO2 introduced from the back of the mask to simulate a large animal attached to the exposure system to ensure that ECO2 (end-tidal CO2) values returned to baseline between breathes over a wide range of operating parameters. Upon introduction of the new masks, decreased the mean in vivo dog respiration rates from a mean of 24.9 and 20.0 breaths/min in the males and females to 16.9 in both sexes. Decreases in respiration rates in mature primates were also observed from 42.4 and 43.3 breathes/min in the male and females to 34.6 and 40.8. The mean respiratory values decreased from 71.4 breathes/min in the males and 77.5 in the females to 64.7 and 64.1 respectively. These decreases in the respiration rate was also accompanied by anecdotal visual dosing observations that the animals appear noticeably calmer especially primates and thus improving animal welfare. This personalized approach to mask fitting and design has been adopted for all future studies.

2020 Comparison of Deposition Efficiency and Uniformity of Monodisperse Solid Particle Deposition in Two Air Liquid Interface (VITROCELL 24/48 and AMES 48) In Vitro Exposure Systems with Computational Fluid Dynamic (CFD) Predictions

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For toxicological assessment of inhalable chemicals, in vitro exposure systems that enable aerosols to be delivered directly to the apical surface of respiratory cells (air liquid interface; ALI) provide a more realistic exposure method than traditional submersion in vitro cultures. Quantitative aerosol dosimetry (delivered dose) is critical for interpretation of biological results generated from these ALI in vitro exposure systems and potential extrapolation to humans in the gut microenvironment available ALI in vitro exposure systems (VITROCELL® 24/48 and AMES 48) particle deposition efficiency and uniformity of deposition across the cell culture inserts and petri dishes were experimentally quantified and compared with CFD predictions. Four diameters of monodisperse fluorescent particles (0.51, 1.1, 2.1, and 3.2 μm mass median aerodynamic diameter) were used and experimentally measured. For the VITROCELL® 24/48 exposure system, experimentally measured particle deposition efficiency ranged from a mean (N= 3 runs) of 0.013% to 0.86% as a function of particle diameter. Variability in the uniformity of particle deposition across the cell culture inserts was observed and ranged from 40% to 150% of the mean number of particles. There was good agreement between experimentally measured and CFD predicted particle deposition efficiency and uniformity of particle deposition for the VITROCELL® 24/48 exposure systems. For the VITROCELL® AMES 48 exposure system, three different sampling flows (5, 10, and 20 cc/min) were evaluated. The 10 cc/min sampling flow provided the most consistent number (65 - 135% of mean number of depositing particles), regardless of particle size. Experimentally measured deposition efficiency (10 cc/min flowrate) ranged from a mean (N=3 runs) of 0.07% to 0.43% as a function of particle diameter. Quantitative aerosol dosimetry in these two ALI exposure systems enables improved experimental design and extrapolation to human exposures.

2021 HIF-1α and IL-1β Are Two Key Events of the Lung Inflammation and Fibrosis Induced by Particles Used in Li-Ion Batteries

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Rechargeable Li-ion batteries (LIB) offer undeniable advantages compared to other technologies and are increasingly used worldwide. They contain micrometric and low solubility particles, consisting of toxicologically relevant elements, implying a potential for inhalation exposure in occupational settings. New proposed applications such as printable or spray-paintable batteries might also expose consumers. As the health hazard of these materials is not documented, we performed the first study on the respiratory hazard of 3 leading LIB components (LiFePO4, or LFP, Li2TiO3, or LTO, and LiCoO2 or LCO) and investigated their mechanisms of action. Lung responses were assessed in mice after oro-pharyngeal aspiration of LIB particles or crystalline silica used as reference. Acute inflammatory lung responses and oxidative stress were recorded with the 3 LIB particles and silica, LCO being the most potent. Inflammation persisted 2 m after LFP, LCO and silica, in association with fibrosis in LCO and silica lungs. Only LCO stabilized hypoxic-like factor (HIF)-1α, a pro-inflammatory and carcinogenic transcriptional factor stabilized by Co ions, after 3 d [1]. In view of the large variety of existing and in development LIB particles, their increasing production, use and disposal, a predictive in vitro assay of their lung toxicity and potential for occupational health risks. By inhibiting HIF-1α or IL-1β responses, we identify these 2 markers as key events of LCO lung toxicity. The in vitro and in vivo study of a large range of LIB particles with different % of cobalt allows us to confirm the predictive value of the in vitro assay for large number of LIB particles. We conclude here that particles used in LIB represent a respiratory hazard. Exposure to LIB particles should, therefore, be strictly controlled in occupational settings. LCO was more potent than crystalline silica to induce inflammatory and fibrotic responses. IL-1β and HIF-1α stabilization represent key events in the lung toxicity of LIB particles and appear useful biomarkers to compare the large number of LIB particles in development or on the market. [1] V. Sironval, et al., Archives of Toxicology 92, 1673-1684 (2018).

2022 Timing of Rat Gestational High-Fat Diet and Sex Determine Increased Susceptibility to Allergic Responses in Offspring

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We previously showed offspring from Long-Evans rat dams given a high-fat diet (HFD) before, during, and after pregnancy had increased pulmonary and metabolic responses to acute ozone exposure. Increased susceptibility to allergy and asthma may also result from maternal HFD consumption. To determine if there is a window of susceptibility to allergic responses in offspring due to HFD consumption during gestation, dams were fed a control diet (CD) throughout gestation, HFD on gestation days (GD) 1-11 followed by CD on GD 12-22 (HFD/CD), CD from GD 1-11 followed by HFD on GD 12-22 (CD/HFD), or HFD throughout gestation (HFD). Male and female offspring (average 16 weeks old on day 0) were sensitized and challenged intranasally with increasing concentrations of house dust mite (HDM) antigen on days 0 and 7 and challenged i.n. on day 21. Non-allergic rats received saline vehicle only on sensitization days. Respiratory responses to methacholine aerosol (0, 10, 20, and 40 mg/ml saline) were assessed by whole body plethysmography 2 days after challenge. Enhanced pause (PenH), an indicator of labored breathing, was significantly increased in female HDM-allergic HFD and CD/HFD groups compared with the female allergic CD group during 40 mg/ml MCh exposure. There were no differences among male groups, and other indices of respiratory function were not affected by maternal diet in non-allergic or HDM-allergic groups. Alveolar macrophages not treated ~99% of bronchoalveolar lavage fluid (BALF) cells in all non-allergic groups. BALF neutrophils, eosinophils, and lymphocytes together comprised ~6% (females) to ~12% (males) of total cells in HDM-allergic groups, indicating mild allergic inflammation, but there were no effects of maternal diet on inflammatory responses. Maternal diet also had no influence on BALF protein and albumin concentrations and inflammatory cytokine levels in allergic and non-allergic groups. In contrast, BALF γ-glutamyl transferase, a marker of lung injury, was significantly increased (1.8-1.9-fold) in both
non-allergic and HDG-allergic CD/HFD and HFD male groups compared with CD males. Together these data suggest increased susceptibility to allergic airway hyperresponsiveness in female offspring due to maternal HFD through- out or in the latter half of pregnancy. Greater lung injury in male offspring due to the same maternal diets may predispose to increased susceptibility to air pollutants. This abstract does not reflect US EPA policy.

2023 Evaluation of Diacetyl Mediated Pulmonary Toxicity in Physiologically Relevant Air-Liquid Interface Models of Human Primary Bronchial Epithelial Cells


Diacetyl, an α-diketone, is used extensively in artificial flavorings due to its butter like aroma. Exposure to diacetyl vapors is associated with the development of bronchiolitis obliterans in workers of microwave popcorn and flavor manufacturing industries. Therefore, we aimed to investigate diacetyl induced pulmonary toxicity using physiologically relevant air-interface model (ALI) models of human primary bronchial epithelial cells (PBEC). The PBEC-ALI models were exposed to clean air (sham) or to 1, 3, 10 and 30 ppm of diacetyl vapor for 30 minutes using an in-house developed exposure system. Cell viability was analyzed with LDH-assay. Transcript expression of pro-inflammatory (CXCL8, IL6, IL1B, TNF, and NFκB1), oxidative stress (HMOX1, SOD3, G5A1 and GPX3), tissue injury/repair (MMP9/TIMP1) and anti-protease β-defensin (SLPI) and H2O2 markers were assessed 6 and 24h post-exposure using qRT-PCR. Secretion of CXCL-8 and MMP-9 were measured in both basal media (BM) and apical media (AM) using ELISA. More than 90% cells were viable after exposure to both sham and diacetyl vapors. Significantly increased secretion of MMP-9 was detected 24h post-exposure compared to 6h post exposure group in both AM and sham (P<0.05). However, secretion of CXCL-8 remained unaltered. Significantly altered expressions of pro-inflammatory (NFκB1, TNF, IL6), oxidative stress (GPX3, G5A1, PTGES2) and tissue injury/repair (MMP9/TIMP1) markers were more pronounced at 24h post-exposure to diacetyl compared to 6h post exposure. Transcript expression of anti-protease marker (SLPI) was significantly increased at 24h post-exposure to 1ppm diacetyl, whereas at 6h post-exposure to 30 ppm diacetyl results in reduced expression of SLPI. Our study shows that diacetyl exposure results in significantly increased expression of pro-inflammatory, oxidative stress, anti-protease, and tissue injury/repair markers. Altered expression of these markers may play a pivotal role in diacetyl modulated pulmonary toxicity. Further, the physiologically relevant ALI models with exposure system used here are a highly suitable in vitro approach to assess occupational exposure induced health hazards.

2024 Docosahexaenoic Acid Supplementation Effectively Treats Toxicant-Triggered Autoimmunity in Lupus-Prone Mice


Systemic lupus erythematosus (lupus) is a chronic and debilitating autoimmune disease that affects approximately 1.5 million people in the United States. Pulmonary exposure to crystalline silica dust (cSiO2) has been implicated as an environmental trigger of lupus. We have recently reported that consumption of docosahexaenoic acid (DHA) prevents cSiO2-triggered autoimmunity in lupus-prone mice. Specifically, DHA supplementation suppressed cSiO2-induced 1) pulmonary ectopic lymphoneogenesis, 2) cytokine and autoantibody responses in both bronchoalveolar lavage fluid (BALF) and plasma, and 3) glomerulonephritis in the female NZBWFI mouse model. The present study is designed to address the question: Can DHA supplementation alone, or in combination with cSiO2, ameliorate the development of these lupus-like symptoms? To test this hypothesis, we induced disease in only C57BL/6 female mice using a single, low dose nCuOCOOH nanoparticles. Additionally, because response to transition metal nanoparticle was as highly enriched GO term (FDR BE-13) from the subset of 48 highly overlapping DE genes, these genes may represent biomarkers to a potentially large variety of metal/metal oxide nanoparticles.

2025 Natural History of Inhaled Sulfur Mustard Poisoning in Rats

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In humans, inhalation of sulfur mustard (SM, bis (2-chloroethyl) sulfide), causes acute lung injury which progresses to fibrosis. Herein, we characterized the manifestations of SM-induced pulmonary injury in rats. Spontaneously breathing male Wistar rats were anesthetized, intratracheally intubated, and exposed to 0.4 mg/kg SM by vapor inhalation. Animals were euthanized 3, 7, 16 and 28 d after and bronchoalveolar lavage (BAL) and lung collected. At 3 d post SM, pulmonary edema, inflammatory cell accumulation, thickened alveolar septal walls and ulceration of bronchial epithelium were observed in histologic sections. Expression of HO-1 and iNOS were also markedly upregulated in the lung, along with mucin, fibrinogen and COX-2, consistent with SM-induced oxidative and nitrosative stress, lung injury and inflammation. This was associated with increased in BAL cell protein content and levels of HMGB-1, RAGE, MPO, and SP-D, demonstrating alveolar epithelial and inflammatory cell involvement in lung injury. Gene expression changes were also noted in the lung at 7 d post SM, but at reduced levels. At 16 d post SM, lung HO-1 and mucin expression were increased, relative to 7 d. Additionally, bronchial epithelial- and mesothelial-hyperplasia/hyperplasia, interstitial fibrosis and dysregulated epithelial repair were evident. At 28 d post SM, a secondary increase in inflammatory cell infiltration in BAL, cilia, airway and bronchi, and MPO, RAGE, SP-D and fibrinogen levels, as well as HO-1 and COX-2 expression was observed which was comparable to or greater than 3 d post SM. This correlated with the appearance of proteinaceous bronchiolar and alveolar exudate entrapping inflammatory cells, diffuse squamous metaplasia, aberrant bronchial epithelial repair and multilayered interstitial and peribronchial fibrosis. These data demonstrate a similar pathologic sequela of events in rats and humans following SM exposure. Development of a rodent model of injury that reflects human exposure will be useful for the identification of efficacious therapeutics for mitigating SM-induced acute injury and fibrosis. NIH Grants: US4AR055073, R01ES004738, P30ES050522.

2026 Molecular Signature of Asthma-Enhanced Sensitivity to Aerosols of Pristine and Carboxylated CuO Nanoparticles, Identified in 3D Cell Models

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More than 5% of any population suffers from asthma, and these individuals may be more sensitive to nanoparticle aerosols than the healthy population. There is as yet a paucity of data on the molecular underpinnings and mechanisms involved. We therefore investigated global transcriptic responses of human bronchial epithelial cell (BE-2) models to pristine and carboxylated CuO nanoparticles (CuOCOOH) in the lung lipids. Pulmonary exposure to 0.4 mg/kg SM by vapor inhalation. Animals were euthanized 3, 7, 16 and 28 d after and bronchoalveolar lavage (BAL) and lung collected. At 3 d post SM, pulmonary edema, inflammatory cell accumulation, thickened alveolar septal walls and ulceration of bronchial epithelium were observed in histologic sections. Expression of HO-1 and iNOS were also markedly upregulated in the lung, along with mucin, fibrinogen and COX-2, consistent with SM-induced oxidative and nitrosative stress, lung injury and inflammation. This was associated with increased in BAL cell protein content and levels of HMGB-1, RAGE, MPO, and SP-D, demonstrating alveolar epithelial and inflammatory cell involvement in lung injury. Gene expression changes were also noted in the lung at 7 d post SM, but at reduced levels. At 16 d post SM, lung HO-1 and mucin expression were increased, relative to 7 d. Additionally, bronchial epithelial- and mesothelial-hyperplasia/hyperplasia, interstitial fibrosis and dysregulated epithelial repair were evident. At 28 d post SM, a secondary increase in inflammatory cell infiltration in BAL, cilia, airway and bronchi, and MPO, RAGE, SP-D and fibrinogen levels, as well as HO-1 and COX-2 expression was observed which was comparable to or greater than 3 d post SM. This correlated with the appearance of proteinaceous bronchiolar and alveolar exudate entrapping inflammatory cells, diffuse squamous metaplasia, aberrant bronchial epithelial repair and multilayered interstitial and peribronchial fibrosis. These data demonstrate a similar pathologic sequela of events in rats and humans following SM exposure. Development of a rodent model of injury that reflects human exposure will be useful for the identification of efficacious therapeutics for mitigating SM-induced acute injury and fibrosis. NIH Grants: US4AR055073, R01ES004738, P30ES050522.
**2027 Comparative Toxicities of 1,1′-Methylenedioxy(4-[(hydroxylimino)methyl]pyridinum) (MMB4) at Rat and Rabbit Diaphragm Neuromuscular Junctions**


1,1′-methyliden(4-[(hydroxylimino)methyl]pyridinium) (MMB4) is a leading candidate for next-generation nerve agent treatment. Although MMB4 appears clinically nontoxic at up to 400 mg/kg in rodents, MMB4 has been shown to cause respiratory arrest in rabbits at doses exceeding 200 mg/kg. To determine the mechanistic basis for MMB4 toxicity, we evaluated concentration- and use-dependent effects of MMB4 on diaphragm function by measuring ex vivo nerve-elicited isometric twitch, tetanic contraction strength, and synaptic transmission via intracellular electrophysiology in female New Zealand white rabbits and Sprague Dawley rats. Results from muscle function studies indicate that MMB4 more potently inhibits diaphragm muscle contraction in rabbit than in rat. Intracellular recording studies demonstrate that MMB4 causes a dose-dependent reduction in rat end plate potentials (EPP) and miniature end plate potential (mEPP) amplitudes, with compensatory increases in quantal content. While concomitant decreases in rabbit EPP and mEPP amplitudes were also observed, quantal content remained unaffected by MMB4. These data indicate that the increased toxicity of MMB4 at rabbit versus rat phrenic neuromuscular junctions results from (1) a lack of compensatory synaptic homeostatic mechanisms in rabbit; (2) inhibition of compensatory mechanisms in rabbit; or (3) augmentation of compensatory mechanisms in rat. We also observed increased EPP and mEPP half-widths in rat and rabbit recordings, indicating that MMB4 inhibits AChE. Finally, while there was no evidence of use-dependent effects of MMB4 in rabbits, pronounced tetanic fade was seen in rats, suggesting the potential antagonism of presynaptic nicotinic acetylcholine receptors. Collectively these results demonstrate that oximes such as MMB4 can have a multifactorial effect on cholinergic synaptic transmission and provide an explanation of a potential mechanism for the increased sensitivity of rabbits versus rats to MMB4 overdose.

**2028 Development of a Computational Model for the Transient Receptor Potential Vanilloid Subfamily Type 1 Protein (TRPV1)**


Sensory irritation, perceived irritation in the nose, throat and eyes, is a common manifestation of exposure to irritants and is frequently used as a critical effect in setting airborne occupational exposure limits (OELs). The transient receptor potential vanilloid subfamily type 1 protein (TRPV1); capsaicin receptor) is an important biological target for potential sensory irritation but there are no available in silico or in vitro predictive models. The goal of our work was to mine existing public data and develop a computational model that predicts the likelihood an unknown compound will or will not interact with TRPV1. We compiled a master database of >30000 compounds known to interact with TRPV1 and >8500 compounds that do not. We developed a random-forest machine-learning prediction model that derives, predicts and analyzes structural fingerprints, conserved scaffolds and protein-docking binding energies. Our machine-learning prediction model that derives, predicts and analyzes structural fingerprints, conserved scaffolds and protein-docking binding energies. Our model showed very high sensitivity (95%) and balanced accuracy (82%), with a specificity of 68%. Furthermore, analysis of binding energetic from docking revealed TRPV1 active substances to have significantly higher binding energies relative to controls (p<0.0001). In conclusion, we have built the only available computational model for TRPV1, an important biological target. We can now use our model to screen in vivo databases for potential involvement of this target.

**2029 A Read-Across Study on Diketones**

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The alpha-diketone diacetyl (2,3-butanedione) is used as butter flavorant in the microwave pop corn industry and suspected to cause a rare obstructive pulmonary disease (popcorn lung). Smoking is also a potential route of exposure to diacetyl and the potentially less toxic alternative 2,3-pentanediol. In this case study we are developing new-approach methodologies (NAMs) to reduce the uncertainty of read-across pre-dictions. Primary bronchial epithelial cells were exposed to the alpha-diketones diacetyl, 2,3-pentanediol and 2,3-hexanediol or the beta-diketone 2,4-pentanediol under air-liquid interface (ALI) conditions using the P.R.I.T. ExpoCube device. This unique exposure device provides a highly efficient exposure situation by preventing contact between the test compound and the culture medium. Primary human bronchial epithelial cells (PBECs) from tumor-free lung tissues from four donors were differentiated in airway epithelium at ALI conditions. Test atmospheres were generated by evaporation of the volatile test compounds and diluted in clean air. FT-IR spectroscopy enabled online analysis of the exposure concentration. PBECs were exposed for 1h once or repeatedly on three consecutive days. Cellular viability was measured by LDH-leakage and monolayer integrity by measuring the transepithelial electrical resistance (TEER) 24h after the final exposure. Exposure concentrations ranged from 100 to 1840 ppm (diacetyl) and from 50 to 5000 ppm (other diketone analogues). Lowest observed adverse effect levels (LOAELS) were lower after repeated exposure compared to the single exposure. Read-across derived with alpha- and beta-diketone specific toxicity since 2,4-pentanediol displayed significantly lower cytotoxic effects in comparison to its analogues. In conclusion, in vitro testing of volatile gases enabled ranking of test compounds with regard to inhalation toxicity. Moreover, further comprehensive evaluations may adequately predict specific lung toxicity of structurally related compounds. Acknowledgement: This project received funding from the European Union’s Horizon 2020 research and innovation programme (grant agreement No 681002).

**2030 Effects of Low Level Hydrogen Sulfide Exposure on the Pathogenicity of Influenza A Virus Pathogenicity in a Swine Model**

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Hydrogen sulfide (H2S) is a toxic gas that affects the respiratory, cardiovascular and central nervous systems. In intensive swine confinement operations, H2S is a hazard for both humans and swine. It has been shown that H2S is an upper and lower respiratory tract irritant. Influenza A virus (IAV) is a zoonotic disease of public health significance. However, the effects of repeated low level exposures of H2S on the pathogenicity of IAV or toxicity of H2S have not been investigated. We hypothesized that repeated exposure to low concentrations of H2S increases the pathogenicity of IAV. To test this hypothesis, thirty pigs were exposed to H2S concentrations ranging from 0 to 50 ppm for 6 hours daily for 12 days; five controls were exposed to breathing air (BA). Pigs were exposed for 7 days before challenge with approximately 3×10^5 TCID50/ml H3N2 IAV (C) or given placebo (NC) on day 0. The experimental design was as follows: Group 1 BA (0ppm H2S)/NC; Group 2 0ppm H2S/C; Group 3 0.5ppm H2S/C; Group 4 5ppm H2S/C; Group 5 50ppm H2S/NC; and Group 6 50ppm H2S/C. Pigs were weighed upon arrival, and on days 0 and 5 post-inoculation (dpi). Body temperature and clinical observations were collected daily including coughing, respiratory distress, lethargy and eye irritation. All pigs were euthanized after exposure on dpi 5. The lungs were removed, weighed, and scored for percent lesion severity. Sections of lungs were collected for histopathology and electron microscopy. Preliminary results indicate that H2S at 50ppm/NC reduced growth rate compared to other groups. Group 3 pigs experienced the most significantly elevated body temperature. Clinically, pigs exposed to H2S and influenza exhibited significantly more severe clinical signs compared to inoculated groups without H2S exposure. Grossly, pigs in group 1 and 2 had ‘1’ and ‘2’ lesions that do not. We developed a random-forest machine-learning prediction model that derives, predicts and analyzes structural fingerprints, conserved scaffolds and protein-docking binding energies. Our model showed very high sensitivity (95%) and balanced accuracy (82%) with a specificity of 68%. Furthermore, analysis of binding energetic from docking revealed TRPV1 active substances to have significantly higher binding energies relative to controls (p<0.0001). In conclusion, we have built the only available computational model for TRPV1, an important biological target. We can now use our model to screen in vivo databases for potential involvement of this target.

**2031 In Vitro Phenotypic Profiling of Diacetyl and Other α-diketones in Human Lung Cells**


Diacetyl is an α-diketone (DK) occurring naturally in many foods, beverages, and dairy products. It is also commonly used as a food additive. Occupational exposures to diacetyl may lead to bronchiolitis obliterans. Therefore, other DKs with longer sidechains, such as 2,3-pentanediene (PD), have been proposed as diacetyl substitutes. Previous animal studies found that PD may also induce bronchial fibrosis. However, it is unclear that, in the human lung cells, if these other DKs induce similar or different cellular effects as diacetyl. We have previously developed an in vitro pulmonotoxicity assay based on High-Throughput In Vitro Phenotypic Profiling (HITTox) of a human bronchial epithelial cell line, BEAS-2B. We used machine learning to identify a high-
ly-predictive phenotypic feature measuring the spatial cross-correlation of cellular DNA and yH2AX stains (F1), which can achieve 88.8% balanced accuracy (84.6% sensitivity, 93.0% specificity) in predicting the in vivo pulmonary toxicity of 33 reference chemicals. Here, we present a study to use the assay to compare diacetyl and three other Dks: PD, 2,3-hexadiene, and 2,3-heptadiene. Cells were treated with the chemicals at seven concentrations (0.87 μM - 2 mM) for 16 hours; and fixed, stained, and imaged with Hoechst 33342, phalloidin, anti-yH2AX, and CellMask®stains. For diacetyl, we also performed an alkali Comet assay at 4 and 16 hours to measure DNA strand breaks (DSBs), and a resazurin assay at 72 hours to measure cell viability. We found that 2 mM diacetyl significantly increased the value of the predictive feature (mean log-ratio of F1 = 0.026 ± 0.036, P = 0.003 ± 0.039, t-test). The Comet assay revealed that 2 mM diacetyl induced DSBs at 4 hours (mean % tail DNA = 0.81, P = 0.02, t-test), but not at 16 hours (mean % tail DNA = 0.15, P = 0.58, t-test). Our results showed that diacetyl and other Dks induce DNA damage in human bronchial epithelial cells. Diacetyl causes early DSBs, which may be repaired later. However, the cells would still die eventually. Diacetyl is more active than other Dks with longer sidechains, but all the tested Dks induce similar effects to the lung cells, and predicted to be positive by our HIPPTox lung assay.

2032 A Method for Determination of Tracheobronchial Airway Geometries from Four Different Strains of Mice
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1PMRI Research Laboratories Pte. Ltd., Singapore, Singapore; 2Altria Client Services LLC, Richmond, VA; 3Philip Morris International RD & R, Neuchatel, Switzerland; and 4Synopsys, Exeter, United Kingdom. Sponsor: J. Hoeng, American College of Toxicology

Accurate lung morphology is fundamental for predicting aerosol dosimetry. Currently, lung morphology is only available for 2-3 strains of mice (B6C3F1, BALB/c, and A/J). Based upon in situ prepared silicone rubber mouse lung casts, a complete process including their micro-CT scanning, segmentation, and automated algorithmic processing enabling the determination of airways geometries was developed for four strains of mice (BALB/c, A/J, ApoE/- and C57BL/6). Silicone rubber lung casts were prepared in situ from 20 ApoE/- and C57BL/6 mice. The cured mouse lung casts were manually inspected for casting quality and manual morphometry measurements were performed (tracheobronchial generations 1-6) on selected lung casts prior to high resolution micro-CT scanning. Micro-CT scanning of existing in situ lung casts from BALB/c and A/J mice were also performed. Micro-CT Images were then segmented to reconstruct a 3D model of the individual lung casts. A skeleton of each processed lung cast was automatically created by shrinking the 3D model of each airway to its centerline. Algorithms were developed for automatic detection of possible skeleton exceptions like closed loops, trifurcations and isolated nodes to be subsequently manually resolved. Finally, the skeleton was automatically measured extracting major airway morphometry characteristics (e.g. airway generation number, length, diameter, bifurcations angles, and angle to gravity). The automated measurement procedure was performed (tracheobronchial generations 1-6) on selected lung casts prior to the 3D model of each airway to its centerline. Algorithms were developed for automatic detection of possible skeleton exceptions like closed loops, trifurcations and isolated nodes to be subsequently manually resolved. 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for 7 days and maintained in room air or placed in oxygen (85% O2) for 7 days or 14 days. After hyperoxia exposure, the newborn animals were sacrificed and lung and liver tissues were collected for histopathology, morphometry, mRNA levels, protein content and enzyme activities of CYP1A1/1A2/1B1, and major angiogenesis genes. Newborns exposed to prenatal BP displayed more severe lung injury at PND7 and PND14 as compared to other groups with increased pulmonary edema, abnormal alveolarization and vaso-obliteration. Postnatal VA treatment of these newborn rats ameliorated the lung injury due to both postnatal hyperoxia and prenatal exposure to BP. These results indicate that VA attenuates the BP mediated and hyperoxia induced lung injury. There was a significant increase in liver CYP1A1/1A2 and lung CYP1B1 mRNA levels in VA treated hyperoxia group compared to others. Interestingly, VEGF protein was also significantly increased in lungs after VA treatment in hyperoxia and BP exposed group. These results suggested that VA attenuated hyperoxia and BP mediated neonatal lung injury by CYP1A/B1 dependent mechanisms and by activating VEGF as a key paracrine factor with cytoprotective and anti-inflammatory protective effects. It is possible that VA treatment imparted a protective due to the preservation of the lung alveolar and vascular development. This study could have major implications for the prevention or treatment of neonatal lung diseases due to hyperoxia and/or maternal environmental exposures to BP.

2036 Human Multi-Organ-Chip Co-Culture Approach of Bronchial Airway and Liver Models for Substance Exposure Studies

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At present, the predictability of cell culture-based methods to assess the effects of substances on the human body is limited, as they fail to emulate organ complexity and cross-talk. Biology-inspired microphysiological systems, such as TissUse’s Multi-Organ-Chip (MOC) platform, provide pre-clinical insights into absorption, distribution, metabolism, and toxicity of substances on a systemic level using human tissues. Here we describe a co-culture of a human liver equivalent based on the HepaRG™ cell line, combined with human stem cell-derived airway epithelial cells and a bronchial equivalent based on the MuclAir™ model. The co-culture has been designed to elucidate the toxicity of inhaled compounds and to predict their effects and metabolism in a trans-organ environment. To address this challenge, a new MOC design and adequate co-culture conditions were established. Compared with an earlier version, the MOC was redesigned to optimize medium supply as well as to allow better oxygenation of the organ models. The tissue constructs were integrated in separate culture compartments of the closed circulatory perfusion system, interconnected by microfluidic channels, for up to 14 days. Tissue viability and homeostasis could be demonstrated by adenosine triphosphate-based cell viability assay, lactate dehydrogenase release, and metabolic profiling. Oxygenation was monitored over the culture period using an oxygen sensor (PreSens). Integrity and function of MuclAir™ tissues were additionally evaluated by histological analysis and measurements of trans-epithelial electrical resistance and cilia beat frequency. Furthermore, immunohistochemistry, gene expression analysis, and albumin secretion quantification verified liver tissue function in the course of the co-culture. In summary, the new MOC setup enables exposure studies of inhaled substances in order to investigate their toxic effects on a trans-organ level, emulating systemic substance effects on the human body.

2037 A Multi-tiered In Vitro Approach for Sensitive and Predictive Respiratory Safety Assessment

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The chance to successfully bring a novel drug candidate to patients is highly dependent on its safety profile. By integrating predictive toxicology in the drug discovery process, we aim to timely identify and mitigate safety concerns. That strategy relies in part on the employment of in vitro assays that predict clinical safety and provide mechanistic understanding of drug-induced toxicity. The impact of that approach on successful progression of novel drug candidates has become evident, but there are areas for improvement. In the hunt for novel treatments of respiratory diseases, there is a need for universally accepted in vitro strategies to assess drug-induced respiratory toxicity. To address this gap, we developed a multi-tiered strategy to identify and quantify drug-induced effects on airway epithelial integrity, cellular differentiation, inflammatory responses and transcriptomic signatures. To assess effects on airway epithelial differentiation, we developed a confocal imaging assay in which primary human bronchial epithelial cells differentiated on an air-liquid interface (HBEC-ALI) are simultaneously assessed for the expression of acetylated tubulin (ciliated cells), MUC5AC (Goblet cells) and cytokeratin 5 (basal cells) by imaging directly through the insert. We have used this assay to demonstrate loss of cilia and overproduction of MUC5AC in HBEC-ALI exposed to respiratory toxicants. Alongside, inflammatory responses could be induced by increased cytokine secretion. In a separate assay, epithelial barrier integrity was assessed using high-throughput imaging of Calu-3 cells. We could distinguish respiratory toxicants (n=6) from non-toxicants (n=7) by quantifying the tight junction protein ZO-1 at earlier timepoints (48h vs 12 days) and at 20-fold lower concentrations than those required for cell viability studies. Next, we continued our efforts in transplanted Electrical Resistance in HBEC-ALI. Finally, using a novel transcriptomic signature of 68 genes in Calu-3 cells, we achieved 94% predictivity in distinguishing between lung irritants (n=7) and non-irritants (n=11). In summary, we present a novel in vitro assay toolbox for sensitive and predictive assessment of drug-induced respiratory toxicity.
molecule, AE001, was well tolerated and there were no adverse clinical observations or systemic effects detected by assessment of clinical pathology parameters or following histopathological examination of all major organs. The respiratory tract were examined histologically with no indications of local toxicity. The stability of the absorption-enhancer in dose formulation solutions up to 50 mg/ml was determined in vitro by assessing the biological activity on epithelial cell-adhesion. The absorption enhancer, AE001, was capable of disrupting adhesion of confluent epithelial cells.

2040 Carbon Black and Ozone Co-exposure Present Novel Prospects of Disease Susceptibility


Environmental exposures are inherently mixed in nature; however, all the current exposure limits are based on single toxicant exposures. Even with reduced mass based exposures, the severity or incidence of environmental and occupational diseases has not necessarily been reduced. We hypothesize that environmental mixtures/co-exposures can have differential consequences as compared to individual/single toxicant exposures as ultrafine particles can carry gaseous components of environmental pollution to the deeper lung. We developed a whole-body inhalation system for co-exposure to ozone (O3) and ultrafine carbon black (CB). Mice were exposed to 2.0±0.01 ppm O3 and/or 10±1.4mg/cm3 CB for 3 hours. Particle mobility diameter was 140 nm as measured by scanning mobility particle analyzer (SMPS 3938). Aerosol aero-dynamic diameter of 84 nm was measured by an electrical low pressure impactor (ELPI+). Co-exposure aerosols demonstrated a 31% increase in abiotic oxidative potentials as demonstrated by ferric reducing ability of serum (FRAS) assay and a 6% increase in surface oxygen contents. Ultra deep RNA-seq analysis revealed differentially activated pathways related to development, immune and inflammatory processes. A significant modulation of oxidative free radical formation was measured using immune-spin trapping. A 3-4 fold higher and more persistent lung inflammation was induced by co-exposures. We observed alterations in Tev01, airway hyperresponsiveness, tissue elastance and tissue damping indicating more severe lung function decline in co-exposure groups. In conclusion, our results signify the urgent need to evaluate environmental co-exposures and to revisit permissible exposure levels. Funding: NIH/NIGMS U54GM109442-03 (SH), NIH RO1ES015022 (TRN), NIH HL027339 (SJM).

2041 Effects of Subacute Inhalation Exposure to Multiwalled Carbon Nanotubes in Mice and Rats

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The field of nanotechnology is growing exponentially, with investments totaling more than $25 billion. Multi-Walled Carbon Nanotubes (MWCNTs), a type of engineered nanoparticle, possess superior conductive and mechanical properties, resulting in an increase in their production and use. MWCNT toxicity is affected by a variety of factors, including exposure concentration, size, and dose. Current research shows variable outcomes to MWCNT exposure. The primary objective of this study was to determine the fate and transport of MWCNTs in the lungs, their ability to induce inflammation, and their relative retention in the lungs. Mice and rats were separately exposed by inhalation to target concentrations of MWCNT (0, 0.06, 0.2, or 0.6 mg/m3; n=5/group) for 6 hours/day, 5 days/week, over a period of 30 days. Lungs were analyzed 1 and 5 weeks post-exposure (PE). Bronchoalveolar lavage (BAL), cell differentials of BAL protein concentration, and lung histopathology were assessed. There was a significant increase in the total number of cells in the BAL of mice at 1 week PE in the 0.6 mg/m3 group compared to control. By 5 weeks PE, total number of cells returned to within control values. In rats, there was a significant dose-dependent increase in BAL neutrophils at 1 week PE in the 0.6 mg/m3 treatment group compared to all other groups. By 5 weeks PE, the neutrophilic inflammatory response was attenuated, but a minimal dose response was still observed. Although inflammation subsided by 5 weeks PE, there remained a high retention rate of MWCNTs in BAL at this time point in both species. There was a significant increase in concentration of CXCL1 protein for the 0.6 mg/m3 treatment group compared to control in both species at 1-week PE. No lung histopathology was noted in either species for any doses or time points compared to control. Mice and rats display a dose-dependent inflammatory response to MWCNTs in total number of cells and neutrophils. These values return to control values within 5 weeks PE. Continued retention of MWCNTs in the lungs at 5 weeks PE raises concerns regarding possible lasting effects, such as pulmonary fibrosis or cancer.

2042 Critical Analysis of Diacetyl and Bronchiolitis Obliterans


Diacetyl, a natural component of fermented foods and a butter-flavoring agent, was linked to cluster of purported bronchiolitis obliterans (BO) in a group of workers from a single microwave popcorn plant in 2002. Not all animal toxicity, occupational health, and exposure studies agree that diacetyl can cause BO. We conducted a weight of evidence analysis to assess whether the animal studies predict BO in humans generally and among popcorn consumers. A comprehensive PubMed literature search was performed to identify animal studies with relevant routes of exposure (inhalation, intratracheal instillation, or oropharyngeal aspiration). Identified studies were critically reviewed and organized in a systemic manner (by type of respiratory tract lesions). Results indicated rodent pulmonary lesions occurred mainly in nasal passages, less frequently in the upper respiratory system, and rarely in lower airways; the depth of respiratory tract lesions correlated directly with exposure concentrations, which was consistent with diacetyl’s hydrophilic nature. There is a fundamental disparity between the predominantly nasal/upper respiratory tract lesions caused by diacetyl inhalation in rodents and the deep-lung injury observed in cases of BO. The hypothesis for the pathology discrepancy lies in differences between rodent and human breathing patterns and nasal morphometrics, causing diacetyl to be more efficiently absorbed by the nasal cavities of rats than humans. In rats breathing 1 ppm, modeling predicts the nasopharynx absorbs ~80%, trachea/bronchi regions ~18%, and <2% enters the bronchial region. In nasal breathing humans, the nasal region absorbs ~30%, trachea/bronchial regions ~62%, and ~8% enters the bronchial region. Bronchiolar penetration may approach 24% in humans upon coupling light exercise with exclusive mouth breathing, but our review indicated that the modeling assumptions may not be valid. The recent NTP inhalation studies exposed rats to relatively high diacetyl concentrations and observed no lung lesions in rats exposed up to 25 ppm over 3 months or 12.5 ppm over 2 years (i.e., lung lesions were observed only when rats were exposed to 50 ppm for 3 months or 25 ppm for 2 years; NTP, 2018). We found the animal toxicity datasets, together with occupational and consumer diacetyl exposure concentrations, do not support a link between diacetyl and BO.

2043 Moderate Aspergillus versicolor Inhalation Exposure Triggers Neuroinflammation

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Increasing evidence implicates indoor mold exposure in cognitive deficits in children, a process hypothesized to also occur in adults. However, the mechanisms by which inhaled mold and the associated allergic pulmonary response might impact the brain are unknown. Though neuroinflammation is associated with memory deficits and impaired cognition, the environmental triggers of neuroinflammation are poorly understood and the central nervous system (CNS) pro-inflammatory consequences of inhaling mold spores is largely unknown. To address whether a moderate exposure to a common mold found in damp indoor environments, Aspergillus versicolor (AV), could cause neuroinflammation, 8 week old female B6C3F1/N mice were exposed to filtered air, 1x103 heat inactivated AV spores, or 1x104 live AV spores 2 times per week for 1, 2, or 4 weeks. At 48 H after the final exposure, the neuroinflammation marker profile was assessed by RT-qPCR. Analysis of brain tissue from the 4 week exposure revealed significantly elevated pro-inflammatory markers in response to only the live AV exposure in several brain regions: the olfactory bulb (TNFa), frontal lobe (TNFa, IL-10, and CX3CR1), midbrain, and cerebellum (TNFa and CX3CR1). To discern how early neuroinflammation began in response to AV inhalation, 1 and 2 week exposure samples were tested for TNFa mRNA expression. Interestingly, results demonstrate that heat inactivated AV significantly increased TNFa mRNA levels in the olfactory bulb in the 1 week exposure, but not in the 2 or 4 week exposures. In response to inhalation of live AV, TNFa mRNA levels were significantly elevated in brain tissue from 1 week exposure (olfactory bulb and midbrain) and 2 week exposure (frontal lobe, midbrain, and cerebellum). Taken together, these results demonstrate that inhalation of live AV spores triggers neuroinflammation in several brain regions with 1, 2, and 4 week exposures, suggesting a generalized CNS response. However, heat inactivated spores were shown to exert a CNS pro-inflammatory response in only the initial, early 1 week exposure.
Asthma is an airway disease characterized by airway hyperresponsiveness (AHR), inflammation, and remodeling. Enhanced contractile phenotype of airway smooth muscle (ASM) cells in mediates bronchoconstriction and AHR in asthma. Free fatty acids are emerging as signaling molecules with importance in metabolic and inflammatory diseases. Studies showed that long-chain free fatty acids (FFA) acting through G protein-coupled receptors (GPRC), elicited bronchoconstriction in guinea pig models. Hypothesis: We hypothesized that the free fatty acid receptors FFAR1 and FFAR4 modulate excitation contraction (EC) coupling in human ASM cells to regulate AHR. HASM cells were treated with vehicle (ethanol) or GW9508, a synthetic FFAR1/4 agonist, (1-10µM) for short duration (10 min) or 24 h. Myosin light chain (MLC) phosphorylation was determined in the presence or absence of the contractile agonist carbachol (Cch, 10 uM). In parallel, GW9508-treated HASM cells were loaded with fluo-8 and binding dye and carbachol-induced cellular Ca2+ mobilization was measured using fluorescent microscopy. In short duration (10 min), GW9508 significantly attenuated baseline and Cch-induced MLC phosphorylation (n=3 donors) and significantly induced basal and Cch-induced Akt phosphorylation (n=3 donors). Following 24 h exposure, GW9508 significantly attenuated baseline and agonist-induced MLC phosphorylation (n=3 donors). The short duration (10 min) and 24 h exposure to GW9508 have little effect on Cch-induced Ca2+ mobilization in HASM cells. Our findings show that GW9508 attenuates MLC phosphorylation, a biochemical marker of ASM cell shortening in HASM cells, therefore has the potential to broncho-protect human airways from hyper-reactivity.

Preservation of Xenobiotic Metabolizing Capacity in Airway Cells In Vitro: A Species Comparative Approach Using Cells from Mice, Monkeys and Humans

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In vitro culture of primary airway cells at the air-liquid interface (ALI) preserves ciliary function and mucus production, creating an epithelial monolayer similar to in vivo airways. The preservation of xenobiotic metabolic enzyme activity is not documented in existing in vitro airway cell culture literature beyond gene expression. Naphthalene, which requires bioactivation by cytochrome P450 enzymes (P450s) to cause toxicity, was used to determine the impact of in vitro cellular metabolic capacity on toxicity testing. Human small airway cells were purchased, primary trachea cells from C57Bl6 mice and rhinos ma-caques were isolated, and HBE1 cells were used for baseline analysis. Primary cells were also maintained at ALI for 1 month in vitro. Phase I metabolic potential of cells was defined using gene-expression measured by digital-PCR, enzyme activity using Promega P450-1A2-Glo, and metabolism of naphthalene measured by mass-spectrometry. Phase II metabolic potential was defined by analysis of gene-expression of glutathione synthesis and metabolic enzymes, measurement of baseline reduced and oxidized glutathione using HPLC, and measurement of the glutathione response of cells to naphthoquinones. For P450s and microsomal epoxide hydrolase, in vitro gene-expression was the highest in mouse C57Bl6 and rat HBE1 cells; Mucosal HBE1 cells expressed the highest amount of Gclc (~5X higher than HBE1), Gclm (~50X higher), and GSTM1 (~100,000X higher). GSTP1 expression was 10X lower in mouse ALI cells versus HBE1 cells. Assays of mouse ALI cells for metabolism of 1A2-substrate and naphthalene confirmed maintenance of P450 activity in vitro, while the HBE1 cells were found to lack P450 activity. Relative to the freshly isolated mouse trachea cells, the mouse ALI cells preserved ~30% of the P450-1A2 activity. About half of the naphthalene in the mouse ALI exposure was metabolized to form glutathione-conjugates, whereas HBE1 cells did not generate any metabolites. The source species of airway cells impacts the resulting xenobiotic metabolic capacity in vitro. Direct measurement of enzyme activity and glutathione content in addition to gene-expression is recommended to detect metabolically-derived toxicity in airway cell culture systems as activity is not well preserved in a cell line, even when differentiated. Funding: R01 ES020867, P30 ES023513 and T32 HL007013.

Comparative Study of Multiwalled Carbon Nanotubes and Pro-inflammatory IL-1 Beta Production: The Role of Purification and Surface Functionalization

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With advancements in nanotechnology, the application of multi-walled carbon nanotubes (MWCNTs) in commercial products has been increasingly expanding. Consequently, increase in exposure of humans to MWCNTs has raised questions about their potential risks of such materials. The efforts to characterize specific features of MWCNTs that may potentially be associated with adverse health effects have been challenging as variations in the purification process and surface functionalization alter physicochemical properties. As part of the ERA-NET SIINN project ICONS (’International Collaboration On Nanotube Safety’), we examined the consequence of purification, followed by surface functionalization, on MWCNT-induced production of interleukin 1 beta (IL-1 beta) by human macrophages in vitro and in the lungs of mice in vivo. A library of eight differently purified (chemically or thermally) and functionalized (-COOH or -NH2) MWCNT samples were prepared from a single batch of industrially relevant Nanocyl NC7000. THP-1 monocytes were PMA-differentiated to macrophages (40 ng/mL, 48 hrs) and exposed to 1, 10, 100 µg/cm2 MWCNTs. Cells supernatants were analyzed for IL-1 beta via ELISA. Mice (C57BL6 strain, N=4 per group) were exposed via oropharyngeal aspiration to the same library of MWCNTs at doses of 1.6 and 4 mg/kg. After 3 days, BALF was collected and analyzed via ELISA. THP-1 cells exposed to thermally-purified MWCNTs functionalized with -COOH or -NH2 produced more IL-1 beta than when exposed to NC7000. Thermally-purified MWCNTs functionalized with either -COOH or -NH2 produced greater IL-1 beta in the BALF from mice compared to NC7000, whereas chemically purified MWCNTs functionalized with either -COOH or -NH2 produced less IL-1 beta in BALF compared to NC7000. These data suggest that the purification method used prior to surface functionalization is an important determinant in mediating inflammasome activation and IL-1 beta release as part of the innate immune response to MWCNTs. Funding: Supported by NSF Grant 15-022 and NIEHS Training Grant T32ES007046.

Toxicity Screening of Volatile Chemicals Using a Novel Air-Liquid Interface In Vitro Exposure System

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Traditional in vitro dosing methods require, for example, the addition of particulate matter (PM), PM extracts, or chemicals in dimethyl sulfoxide (DMSO) or water into cell culture medium. However, about 10% of chemicals nominated for study in the US Environmental Protection Agency’s (US EPA) Toxic Substances Control Act (TSCA) chemical substance inventory are insoluble in DMSO, water, or are volatile, thus their toxicity cannot be adequately tested using traditional in vitro dosing methods. To circumvent the difficulties in screening for volatile/insoluble chemicals, we developed a new cell culture exposure system (CCES) that permits cells to be exposed at an air-liquid interface (ALI). The ALI method permits a direct pollutant-to-cell interaction in which the test substance is in its natural state, thus providing a more realistic exposure scenario. This novel system is capable of testing 6 different chemical concentrations simultaneously to generate concentration-response curves. In our on-going study, we use the BEAS-2B cell line and primary normal human bronchial epithelial (NHBE) cells to assess the toxicity of volatile chemicals in the TSCA work plan. We exposed cells for 2 h to six concentrations in half-log dilutions, plus an air (vehicle) control to generate concentration-response curves; 1,3-butadiene, 1-bromopropane, acetaldehyde, acrolein, carbon tetrachloride, dichloromethane, formaldehyde, and trichloroethylene have been tested to date. We assessed cell viability 4 h post-exposure via the CellTiter-Glo Assay while we assessed cytotoxicity by measuring lactate dehydrogenase (LDH) in the basolateral medium. Cell lysates were collected 4 h post-exposure for whole transcriptome targeted RNA-Sequencing (i.e., BioSpyder TempO-Seq™). The objective of this study is to evaluate the capability of the transcriptomic data to identify concentration-dependent changes in mechanism/mode-of-action for volatile/insoluble chemicals. This study leverages the ability of the transcriptomic data to group chemicals by similar bioactivity profiles for potential grouping and read across applications. Our highest doses per chemical induced <20% cytotoxicity, while our lowest doses were targeted to not observe adverse effects. Abstract does not reflect views or policies of the US EPA.
2048 Amiodarone-Induced Lung Injury is Associated with Alterations in Alveolar Macrophages and Mesenchymal Stem Cell Populations in Mice


Amiodarone (AD) is an antiarrhythmic drug that causes pulmonary toxicity in 10-17% of patients which can progress to fibrosis. In these studies, we developed an intratracheal administration model of AD toxicity in mice to analyze the response of pulmonary macrophage and mesenchymal stem cell populations and to assess the efficacy of nitrated oleic acid (OaNO2), a potential anti-fibrotic agent, in mitigating toxicity. Male C57BL/6 mice (8-9 weeks old) were administered vehicle control or AD (0.8 mg/kg intratracheally every 5 d) with and without OaNO2 (50 µg per animal) and euthanized 7 d and 14 d after the initial treatment. Cell numbers and total protein content were enumerated in bronchoalveolar lavage fluid (BAL), and alveolar macrophage (AM) and mesenchymal stem cell (MSC) phenotypes were analyzed in BAL and tissue digest, respectively, by flow-cytometry. Administration of AD resulted in reduced weight gain compared to controls 5 d after AD administration; this was associated with a 2-fold increase in BAL cell number and 1.6-fold increase in BAL total protein at 7 d. By 14 d post AD, changes in body weight, BAL cell number, and total protein were similar to controls. AM (CD45+F4/80+SiglecF+) and CD90+ MSC numbers were similar to controls at 7 d, this was partly mitigated by co-administration of OaNO2. CD90+ MSC numbers were similar to control levels at 14 d. Development of a mouse model of AD induced lung injury will be useful for studies examining a role of AM subpopulations and CD90+ MSCs in lung tissue homeostasis and pulmonary toxicity during the pathogenesis of pulmonary injury and fibrosis. Supported by NIH grants ES047738, HL086821, ES005022, and ES007148.

2049 The Multi-kinase Inhibitor, C374, Perturbs Excitation-Contraction Coupling in Human iPSC-Derived Cardiomyocytes

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In a previous 5-day repeat dose study in telemetry-caged cynomolgus monkeys, the multi-kinase inhibitor, C374, caused a dose related, sustained tachycardia and a negative inotropic and lusitropic effect which developed over the duration of the study leading to cardiac lesions. The current study used human iPSC derived cardiomyocytes (hiPSC-CM) to investigate the direct actions of C374 on in vitro cardiac preparation. Action potential (AP), Ca2+ transient (CaT) and contractility (Cnt) were monitored in hiPSC-CMs (CytoSeek) using CellOPTIQ® (Clyde Biosciences Ltd). C374 (0.3, 1.0, 3.0 or 10 µM) was investigated using two protocols: 1) a time course study of the spontaneous AP and Cnt with recordings at 2, 24 and 48 hours following the addition of compound and 2) the effect of C374 on cells paced at 1.5Hz to separate the compound effects on AP, CaT and Cnt from those that are a consequence of chronotropic effects. For protocol 1, at 2 hours, C374 induced a modest concentration related reduction in beat rate (max 1.3x at 10 µM) and a marked prolongation of relaxation time (max 16x at 10 µM) but no effect on Cnt amplitude indicating negative chronotropic and lusitropic effects. This was also evident at 24h but had returned to control levels by 48h. AP duration was prolonged in parallel with the reduction in spontaneous rate. For protocol 2, in cells paced at 1.5Hz, C374 had no effect on time taken to contract or relax or amplitude of Cnt i.e. no inotropic or lusitropic effects at fixed stimulation rates. However, there was a concentration related increase in the duration of the CaT (max 1.8x at 10 µM) which was not reflected in contractility. In conclusion, C374 elicited chronotropic and lusitropic effects in hiPSC-CMs. Lusitropic effects were directionally similar to the in vivo monkey study, whereas, the chronotropic effects were the opposite. This difference in profile is likely due to the differences in the test systems i.e. cardiac function assessed over 5 days in a monkey vs. function of cultured iPSC-CMs assessed over 2 days indicating different underlying cellular mechanisms in hiPSC-CMs compared to the in vivo system. The observed uncoupling between CaT and Cnt indicates a potential mechanism of action for the functional adverse cardiovascular effects of C374.

2050 Calcium Imaging Assay Using Human iPSC-Derived Cardiomyocyte for Cardiotoxicity Risk Assessment of Inotropic Compounds


In drug development, cardiotoxicity is one of the most common cause of clinical safety failure, and it is important to predict cardiotoxicity in preclinical study. Human iPSC-derived cardiomyocyte (hiPSC-CM) is recently used as a tool for in vitro cardiotoxicity screening. Although a lot of studies reported that hiPSC-CM is useful for the risk assessment of QT prolongation and Torsades de pointes, it is unclear whether or not the other cardiotoxicity risk such as positive and negative inotropy effects can be captured in hiPSC-CM assay based on their pharmacological properties. In this study, we evaluated positive (bay K 8644: Ica+ channel activator, digoxin: Na+/Ca2+ exchanger inhibitor, dobutamine: beta 1 receptor agonist, isoproterenol: beta receptor agonist, levosimendan: calcium sensitizer, omecamtiv mecarbil: myosin activator, and forskolin: cAMP activator) and negative (KB-R7943: Na+/Ca2+ exchanger inhibitor, PI-103: TORC1/2 inhibitor, thapsigargin: sarco/endoplasmic reticulum Ca2+-ATPase inhibitor, and verapamil: Ica+ inhibitor) inotropic compounds using hiPSC-CM by calcium imaging analysis. The hiPSC-CMs were cultured on 96 well plates for 7 days. The Ca2+ transient in hiPSC-CMs was acquired by a high-speed acquisition imaging platform (FDSS/µCell, Hamamatsu Photonics K.K.) with Ca2+ sensitive fluorescent dye (Cal520®). AAT Bioquest, Inc.). The multi-parametric data of calcium fluorescence waveform were analyzed by Spotfire 7.6.1 (PerkinElmer Inc.). In the positive inotropic compounds, bay K 8644, digoxin and forskolin increased calcium amplitude. While KB-R7943 decreased calcium amplitude. Verapamil decreased calcium amplitude. While KB-R7943 decreased calcium amplitude and increased peak width duration, verapamil caused the opposite reaction. These waveform changes were consistent with the intracellular calcium kinetics expected form the pharmacological properties of the compounds. In conclusion, the positive and negative inotropic compounds are detected in the calcium imaging analysis using hiPSC-CM based on their pharmacological properties. Our approach will contribute to the improvement of predictability of drug-induced cardiotoxicity.

2051 Mitoxantrone Prompts Early Energetic and Proteomic Changes in HL-1 Cardiomyocytes and Late Oxidative Stress in Clinically Relevant Doses

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Mitoxantrone (MTX) is used in the treatment of cancer and in multiple sclerosis, with cardiotoxicity being a severe side effect. We aimed to study the cardiotoxicity mechanisms of MTX in murine HL-1 cardiomyocytes. Cells were exposed to MTX (1 and 10µM) for 12, 24 or 48h. Phase contrast microscopy, neutral red uptake and MTT reduction assays showed that MTX induced cytotoxicity as early as 12h. At 12h, intracellular ATP levels were increased and media lactate levels decreased following 10µM MTX, while proteomic analysis of total protein extracts found 40 protein spots with significant relative abundance differences (pH gradients 4-7 and 6-11). Of note, the expression of the regulatory 14-3-3 protein epsilon was significantly increased after MTX. Total glutathione significantly increased following 24h exposure to 1µM MTX, which was dependent on gamma-glutamylcysteine synthetase activity, whereas oxidized glutathione only increased at 48h. At 24h, MTX activity was significantly to decrease proteasome chymotrypsin-like activity in a concentration-independent manner, while increasing caspase 3 activity. These data clearly demonstrate that MTX is able to significantly alter proteome, energetic and oxidative stress homeostasis in cardiomyocytes at clinically relevant concentrations.
Cardiovascular (CV) toxicity is one of the leading causes for drug withdrawal in clinical development over the past decades. Some common assays to assess CV toxicities during drug development include hERG binding assays, QT-interval prolongation, and Torsade de Pointes (TdP) using non-clinical models. For in vitro assessment, human induced pluripotent stem cells (iPSC) derived cardiomyocytes are widely used on multi-electrode array (MEA) for studies of cardiac pacemaking. This approach is supported by US FDA Guideline and Comprehensive In Vitro Proarrhythmia Assessment (CIPA) consortium. The use of iPSC cardiomyocytes on MEA platform is useful for an initial CV toxicities screen but has limitations if one desires to study further physiological effects on cardiac function. The limitations come from the immature state of the cardiomyocytes and the 2D platform, which makes it difficult to study in vivo like cardiac function such as pressure to voltage (P-V) relationship. The 3D technology Biowire™II has improved these limitations by 9 weeks of electromechanical conditionings of the iPSC cardiomyocytes. After 9 weeks of conditionings, iPSC cardiomyocytes cultured on Biowire™II display mature cardiac functions such as increased positive force frequency relationship and post rest potentiation and decreased spontaneous beating. We also employed next generation sequencing to evaluate gene expression levels of previously reported 203 adult cardiac genes among the following five groups: One is adult ventricles, and other four groups are from iPSC cardiomyocytes that are either cultured for 1 week on a plate or Biowire™II. The result suggests that the 9 weeks conditionings, rather than the Biowire™II platform, elevates the expression levels of the 203 genes. To understand the global distribution of whole transcriptome in Biowire™II in comparison with adult ventricles and the traditional 2D culture, further gene expression analysis was conducted using long read sequencing. This allows to identify unique gene sets that were improved only by Biowire™II but not the traditional 2D culture. These changes were confirmed by immunostaining assays. In conclusion, the advanced in vitro cardiac models, Biowire™II, will bridge between traditional in vitro 2D cultures and in vivo non-human animal models and enhance transatability to clinical development.

Waterpipe smoking has become increasingly popular despite its centuries-long history, yet little is known about the long-term impact of waterpipe smoke (WPS) on the cardiovascular system. Methods: To investigate the effects of waterpipe smoke (WPS) on cardiovascular disease, hyperlipidemic, apolipoprotein E deficient (ApoE−/−) male and female C57BL/6 mice (n=5) were exposed to either WPS or filtered air as a control for 5 months. The nose-only exposure was 2 hours per day, 4 days per week followed by 3 days of rest each week. Cardiac physiology was assessed by collecting electrocardiograph (ECG) data using implanted radiotelemetry devices, and the ECG data was further analyzed for heart rate variability (HRV). All animals were allowed over 6 hours of recovery time in their housing to limit the effect of exposure stress on HRV measures. HRV was measured by standard deviation of normal R-R intervals (SDNN), root mean squared of successive differences (RMSSD), and High-Frequency (HF) HRV. SDNN represents overall HRV whereas RMSSD and HF HRV are known to represent the fluctuations in vagal inputs to the heart and beat-to-beat variability. Results: HRV showed an initial period of no difference between control and WPS-exposed groups for 3 to 5 weeks. Following this, marked differences in HRV response to WPS was seen between sexes. Males show a response of increased SDNN, RMSSD and HF HRV on the days of exposure in WPS-exposed animals compared to controls. WPS shows a diminished effect on HRV in males on non-exposure days. In contrast, females showed minimal effect of exposure on HRV for the first 4 months on exposure days, but instead have a latent effect apparent on rest days with no exposure throughout the length of the study. On rest days, a decrease in SDNN, RMSSD and HF HRV is seen in females as compared to controls with the difference between exposure groups growing larger throughout the 5-month study. The disparity between exposure and rest day HRV responses points to a latency in the effect of WPS on the cardiovascular system, particularly in long-term cumulative exposure. This study of WPS exposure and HF in hyperlipidemic mice indicates that WPS influences the cardiovascular system as well as the parasympathetic nervous system in a sex-dependent manner.
Arsenic increases atherosclerotic plaque formation in PCSK9-1 AAV transgenic mice.


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Arsenic toxicity is a global concern to human health and is related to increased incidence of cancer, bronchopulmonary and cardiovascular diseases in exposed populations. Cardiovascular diseases are ranked as the primary cause of death worldwide and the majority of them stem from atherosclerosis, the gradual occlusion of arteries by fibro-fatty plaques. Mice are relatively resistant to the development of atherosclerosis; therefore, several genetically manipulated mouse models have been produced to study this condition. Apolipoprotein E (ApoE)-/- is a widely used mouse model, wherein, the glycoprotein ApoE is knocked out, resulting in an impaired LDL and VLDL clearance and a subsequent atherosclerotic phenotype. Our previous findings have shown that low to moderate arsenic concentrations increase atherosclerotic plaques in ApoE-/- mice and alter plaque components. In order to expand potential models of arsenic-induced atherosclerosis, we tested a newer model of atherosclerosis where adeno-associated virus mediated delivery of PCSK9-1 (AAV-PCSK9) induces hypercholesterolemia by increasing the degradation of LDL receptors, thereby decreasing the hepatic uptake of LDL. Here, we evaluated the effects of arsenic exposure in the AAV-PCSK9 mouse model. C57BL/6 male mice were injected intraperitoneally once with AAV-PCSK9 and the following week, randomized to either tap water or 200 ppb arsenic in the drinking water. All mice were fed standard low arsenic diet (not a high-fat diet). Compared to the controls, the arsenic-exposed AAV-PCSK9 mice displayed higher serum cholesterol and LDL levels accompanied by increased atherosclerotic plaque formation after 13 weeks of arsenic exposure, as determined by gross plaque imaging and Oil Red O lipid staining of aortic arch and sinus. In contrast to the ApoE-/- model, we did not observe significant changes in plaque components (i.e. vascular smooth muscle cells and collagen) in arsenic-exposed versus control groups. To fully assess the utility of this model for arsenic exposure studies, further studies will be carried out in the future. Use of the AAV-PCSK9 model will facilitate the generation of further knock-out models to scrutinize the pathways of arsenic-induced atherosclerosis, where crossing of multiple transgenic models is time-consuming and has resulted in embryonic lethality.

The effect of inhaled multiwalled carbon nanotubes on blood pressure in spontaneously hypertensive rats.


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It is well documented that hypertension is one of the main underlying risk factors for cardiovascular disease and is highly prevalent in the general US population. Considering the large percentage of the population with hypertension and the similarity between the pathogenicity of engineered nanoparticles (ENs) and environmental ultrafine particles, the risk assessment of pulmonary exposure to ENs in subjects with pre-existing hypertension is of great relevance. We reported previously that inhalation of multi-walled carbon nanotubes (MWCNTs) transiently elevated systolic, diastolic and mean blood pressure in rats with normal blood pressure. The present study further investigated the effect of inhaled MWCNTs on spontaneously hypertensive rats. Male normotensive Sprague-Dawley or spontaneously hypertensive Wistar rats were pre-implanted with a telemetry device, and exposed by inhalation to MWCNTs at a concentration of either 5 mg/m^3 or 2 mg/m^3 for 5h/day for three consecutive days. The electrocardiogram (EKG) and blood pressure were recorded in real time by the telemetry system at pre-exposure, during exposure, and 1 day and 7 days post-exposure. Inhalation of MWCNTs at a concentration of 5 mg/m^3 significantly increased both systolic and diastolic blood pressure, and decreased heart rate in normotensive Sprague-Dawley rats during the first day of exposure but these parameters rapidly adapted to continuous exposure. However, inhalation of MWCNTs at a lower concentration of 2 mg/m^3 consistently elevated systemic blood pressure for at least 7 days in spontaneously hypertensive Wistar rats. Our study indicates that the blood pressure of spontaneously hypertensive rats is more vulnerable and less tolerant to pulmonary MWCNT exposure.

Inhibition of TGFβ signaling in rat valvular interstitial cells redirects the activated phenotype.


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Valvular interstitial cell (VICs) are responsible for maintaining the structural integrity of the valve. TGFβ signaling is integral for proper functionality of the VICs and various TGFβ receptor inhibitors have been reported to cause valvulopathy in the rat. It has been speculated that VICs are the cellular target of toxicity of TGFβ receptor inhibitors. We used primary cultures of rat VICs to characterize by cellular, biochemical and molecular methods, early changes in response to inhibition of TGFβ signaling. We found that rat VIC cultures treated with TGFβ present robust stimulation of p-5MAOD3 with effective concentration-responsive inhibition of p-5MAOD3 following treatment with TGFβ receptor inhibitors, indicating that VIC cells are a target of TGFβ signaling. A concentration range of TGFβ inhibitor treatment (0.001-10 μM) was determined to not cause toxicity by apoptosis, necrosis and cellular morphology evaluations. At these sub-toxic concentrations, the VICs presented decreases in ATP production and cellular respiration that may indicate alteration of mitochondrial function. However, there was also a decrease in reactive oxygen species (ROS) suggesting enhanced oxidative stress was not part of the response. Transcriptional evaluation of a series of targets involved in EMT and ECM revealed a concentration-related alteration of EMT markers, TAG2A and Tagln, and ECM targets, MMP2 and COL1A1. Furthermore, a majority of the VIC were of the activated phenotype, positive for alpha-smooth muscle actin (α-SMA) and spindle shaped, whereas other cells were positive for vimentin and had cuboidal or rounded appearance. Following 48-hour treatment with TGFßR inhibitors, a majority of the cells were positive for vimentin with rounded appearance and cells positive for α-SMA presented less organized actin filaments, with concentrated staining near the cell membrane. Taken together, these results suggest the pathogenesis of TGFßR inhibitor-induced valvular toxicities may involve redirection of the activated VIC phenotype to a more mesenchymal-like or quiescent phenotypes that have decreased ability to engage in repair or response to injury.

Statistical power analysis for cardiovascular safety pharmacology studies in dogs.


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One of the major causes of drug attrition is drug-induced effects on cardiovascular system. The ICH S7B guideline describes a non-clinical testing strategy for assessing the potential of a test article to slow ventricular repolarization prior to testing in humans. The quality of the data and the assessment of cardiovascular safety of the new drug would be affected by the study design and data analysis procedure. The purpose of the present project is to investigate the prospective statistical power on number of dogs for detecting a minimum effect size of the maximum effect between the high dose group and the control group of a test article over time for specific cardiovascular endpoints by different pharmacokinetic (PK) patterns using the simulated data. Eighteen previous cardiovascular safety pharmacology studies submitted to US FDA with a 4-by-4 double Latin square (LS) design with 8 beagle dogs using implanted device were selected as the historical data to assess the centrality and dispersion for the simulation. Between- and within-animal variability and other parameters related to different covariance structures across time (CS and AR(1)) were derived, using the repeated measures analysis of covariance. Four representative PK patterns with various maximum effect size over time were used to construct different simulation data. A linear mixed effect model was used to analyze the test article effect over time. In addition, LS designs with 4 and 12 dogs are also examined with the same approach. The power analysis is conducted to the dose response by a testing procedure which involves both trend test and pair-wise comparison at each time point after dosing. The power analysis shows that a minimum of 8% increment in QTcF can be detected with more than 80% power when 8 dogs were used in a LS design with compound symmetric covariance structure; whereas 6% and 13% increment in QTcF can be detected with more than 80% power for 12 dogs and 4 dogs, respectively. The overall false positive rate of the testing procedure to detect the significance of the dose effect is 7.4%. Furthermore, a minimum of 8% increment in QTcF with first-order autoregressive covariance structure; a minimum of 22% increment in heart rate (HR) with compound symmetric covariance can be detected with more than 80% power when 8 dogs were used in a LS design.
Nitrate and nitrite are present in canine feeds due to incorporation of plant materials high in these compounds, as well as use as a preservative for protein ingredients. These nitrogenous compounds have the potential to induce adverse effects such as methemoglobinema, lipid peroxidation and disruptions in cardiovascular function. Conversely, nitrate and nitrite may improve vascular function and reduce hypertension due to conversion into nitric oxide. To examine this relationship, four commercial pet foods and one lab-made diet were evaluated based on varying protein content and price. Seven dogs were randomly assigned and fed each diet for seven days (n=4-7 for each diet).

At the end of each trial, urine, feces, and plasma were collected for nitrate, nitrite and methemoglobin analysis, as well as echocardiography, flow mediated dilation and blood pressure assessed. Nitrate and nitrite concentrations varied significantly among diets, with feed nitrate concentration increasing with higher incorporation of leafy plant products, but also with higher crude protein content. Dietary nitrate was converted into nitrite since only plasma nitrate varied with diet, leading to concomitant significant differences in methemoglobin levels among diets. In contrast, urinary elimination was the primary route for nitrogenous excretion, with plasma nitrite appearing primarily as nitrate in the urine. Urinary nitrate had a statistically significant, negative correlation with methemoglobin (p=0.013, r=-0.54). This could be explained if low urinary nitrate was due to increased methemoglobin as a potential source for nitric oxide production. In support of this hypothesis, there was a weak, positive correlation between methemoglobin and flow mediated dilation. However, potentially negative effects observed were a weak positive correlation between methemoglobin and diastolic pressure as well as an accompanying negative correlation between methemoglobin and stroke volume. Ultimately, results suggest that incorporating higher dietary nitrate and nitrite from non-protein nitrogen sources have the potential to exert beneficial effects on vascular distensibility, working through increases in plasma nitrite and subclinical increases in methemoglobin.

Nitric oxide production. In support of this hypothesis, there was a weak, low urinary nitrate was due to increased methemoglobin as a potential source for nitric oxide production. In support of this hypothesis, there was a weak, positive correlation between methemoglobin and flow mediated dilation. However, potentially negative effects observed were a weak positive correlation between methemoglobin and diastolic pressure as well as an accompanying negative correlation between methemoglobin and stroke volume. Ultimately, results suggest that incorporating higher dietary nitrate and nitrite from non-protein nitrogen sources have the potential to exert beneficial effects on vascular distensibility, working through increases in plasma nitrite and subclinical increases in methemoglobin.

Metallic stents have been proven clinically for their efficacy in reducing the severity of restenosis. However, chronic complications including stent fracture, recurrence of restenosis in stented vessels, very late stent thrombosis and the permanent loss of vasoreactivity encouraging the development of new neoatheroma drive the innovation of fully bioresorbable scaffolds. In this animal study, coronary PILLA scaffolds were evaluated in swine coronary arteries for 2 years. The protocol was approved by AccelLAB animal care and use committee under compliance with the Canadian Council on Animal Care. Yucatan miniswine pigs weighing 8–10 kg were sacrificed at 4–6 months of age to have scaffolds placed intracoronary and assessed for neointima formation using aortic root harvested in ewes implanted in the aorta. Scaffolds were evaluated based on varying protein content and price. Seven dogs were randomly assigned and fed each diet for seven days (n=4-7 for each diet). At the end of each trial, urine, feces, and plasma were collected for nitrate, nitrite and methemoglobin analysis, as well as echocardiography, flow mediated dilation and blood pressure assessed. Nitrate and nitrite concentrations varied significantly among diets, with feed nitrate concentration increasing with higher incorporation of leafy plant products, but also with higher crude protein content. Dietary nitrate was converted into nitrite since only plasma nitrate varied with diet, leading to concomitant significant differences in methemoglobin levels among diets. In contrast, urinary elimination was the primary route for nitrogenous excretion, with plasma nitrite appearing primarily as nitrate in the urine. Urinary nitrate had a statistically significant, negative correlation with methemoglobin (p=0.013, r=-0.54). This could be explained if low urinary nitrate was due to increased methemoglobin as a potential source for nitric oxide production. In support of this hypothesis, there was a weak, positive correlation between methemoglobin and flow mediated dilation. However, potentially negative effects observed were a weak positive correlation between methemoglobin and diastolic pressure as well as an accompanying negative correlation between methemoglobin and stroke volume. Ultimately, results suggest that incorporating higher dietary nitrate and nitrite from non-protein nitrogen sources have the potential to exert beneficial effects on vascular distensibility, working through increases in plasma nitrite and subclinical increases in methemoglobin.

Antiretroviral therapy has prolonged lifespan for HIV-1 patients. However, cardiovascular complications have become one of the most prevalent causes of death among the HIV-1 infected population. Nucleoside reverse transcriptase inhibitors (NRTI) are the backbone of antiretroviral therapy, and the co-factors of emtricitabine (FTC) and tenofovir disoproxil fumarate (TDF/TDF) is commonly used. In prior studies, acute NRTI treatment induced endothelial dysfunction and increased reactive oxygen species production. These effects were 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. We hypothesized that mitochondrial dysfunction, including a compromised/disturbed mitochondrial homeostasis, has a causal role in an endothelial senescence that can exacerbate cardiovascular disease development. First, we evaluated mitochondrial function in human aortic endothelial cells (HAEC) after chronic FTC (10 μM) and/or TDF (10 μM) treatment for 2-14 passages. Senescence associated β-galactosidase staining in NRTI-treated cells demonstrated a higher level of senescence. In late passage HAEC, there was a lower expression of Parkin, a mitophagy modulator, in NRTI-treated cells compared to controls. Using quantitative PCR, mitochondrial DNA copy number exhibited a higher decrement in late passage HAEC treated with NRTI. Second, we treated HIV-1 transgenic mice, Tg26, with FTC (40 mg/kg) and/or TDF (50 mg/kg) for 3 months. Plasma nitrile levels were decreased in the plasma of FTC-treated Tg26 mice. Endothelium-dependent vasodilation of the thoracic aortas of NRTI-treated Tg26 mice was also reduced. High-resolution respirometry analysis in extensor digitorum longus muscle tissues from Tg26 mice showed that FTC/TDF co-treatment decreased mitochondrial respiration in these mitochondria-dominant tissues. Our work suggests that long-term use of NRTI disrupts mitochondrial homeostasis, induces premature endothelial senescence, and impairs vascular function.
**2064 Mitochondrial Alterations May Play a Role in Cardiotoxicity of the Tyrosine Kinase Inhibitor Regorafenib**

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Protein kinases are important enzymes for cell physiology including, cell cycle progression, cell survival and proliferation. Disregulation of these enzymes is associated with cancer. By understanding of these mechanisms in cancer, molecularly targeted and mechanism based therapies have been developed which are mostly targeted to tyrosine kinases and thus are called tyrosine kinase inhibitors (TKIs). Although it has been thought that TKIs are generally well-tolerated for the cancer patients TKIs can lead to serious, fatal adverse effects such as cardiotoxicity. The underlying mechanisms of TK inhibitors induced toxicity is not well understood there is an ongoing effort to clarify them at the molecular level. The aim of this study is to investigate the effects of lenvatinib on H9c2 cardiomyocyte cell model to better understand the molecular mechanisms of TK inhibitors induced cardiotoxicity. It has been hypothesized that the cardiotoxicity of TK inhibitors may be due to mitochondrial toxicity. Cells were treated with 10 µM, 5 µM, 1.25 µM of lenvatinib for 48h and 72h. The cells were examined in aspects of cytotoxicity with methylthiazolyldiphenyl-tetrazoliumbromide (MTT) assay, ATP content assay with luminometry, protein levels with western blot, mitochondrial mass with Mitotracker Green FM and mitochondrial membrane potential (MMP) with JC-1 staining. According to our MTT results; lenvatinib did not cause significant cytotoxicity while with long term exposure cytostatic inhibition occurred at the two drug concentrations 10 µM and 5 µM and the luminometric ATP content assay results showed that the inhibition of the ATP content was 39% and 43%, respectively. Western blotting showed that the expression of Akt-1 was inhibited in the two high doses. P21 protein level increased at 10 µM concentration. Cytochrome c and protein levels of mitochondrial complexes did not show significant difference except for complex I which showed inhibition in 10 µM dose. The results of flow cytometry showed that lenvatinib caused a 44% increase in mitochondrial mass in the 10 µM dose. Lastly, a three fold decrease in the MMP was seen due to depolarization at the highest dose. Considering all these together it can be said that mitochondrial toxicity may be playing a role in cardiotoxicity induced by TK inhibitors.

**2065 Effect of a Cardiotoxic Pollutant-Phenanthrene on the Cardiac Function of Brown Trout (Salmo trutta)**

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Phenanthrene (Phe) is a three-ringed polyaromatic hydrocarbon which is formed from incomplete combustion of hydrocarbons and is also a component of crude oil. Previous studies have shown Phe to be cardiotoxic to marine species. Similar to marine species, this evidence presented here suggest that Phe is cardiotoxic to freshwater salmonids. 2019 SOT Annual Meeting

**2066 In Vitro Investigation of the Antineoplastic Agent Lenvatinib Induced Cardiotoxicity in Terms of Mitochondrial Toxicity**

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Lenvatinib is an oral multitargeted tyrosine kinase (TK) inhibitor which has been approved by US FDA for the treatment of radioiodine refractory differentiated thyroid cancer, unresectable hepatocellular carcinoma, and renal cell carcinoma in combination with everolimus. Cardiac failure is one of the written warnings in the drug label of lenvatinib. Although TK inhibitors are generally considered to be safe antinecancer drugs they can cause adverse side effects such as cardiotoxicity. While the underlying mechanisms of TK inhibitors induced toxicity is not well understood there is an ongoing effort to clarify them at the molecular level. The purpose of the present study is to examine the molecular mechanism of RGF induced cardiotoxicity by using cardiomyocyte cell model. H9c2 cells were exposed to 5 µM, 10 µM, 20 µM RGF for 48h and 72h. Trypan blue evaluation showed that RGF does not affect cell viability and according to MTT assay results RGF decreased mitochondrial activity in a dose and time dependent manner. ATP content which was assayed by luminometer, decreased in all RGF concentrations for both end points. ATP content decreased at 20 µM RGF to 30.66% and 9.41% in 48h and 72h exposures, respectively. Mitochondrial mass evaluated with flow cytometry by labeling with Mitotracker Green FM showed that RGF caused 31.46% decrease in mitochondrial mass compared to the control group. Mitochondrial membrane potential (MMP) by using JC-1 dye showed that RGF treated cells showed higher rates of depolarized MMP (0.91% vs 12.16%). According to the western blot results, Akt-1 levels decreased as a result of kinase inhibition in the two highest doses. Mitochondrial complex III showed a small difference and cytochrome c levels increased as a result of kinase inhibition in the two highest doses. Mitochondrial complex II showed a 44% increase in mitochondrial mass in the 10 µM dose. Lastly, a three fold increase of MMP was seen due to depolarization at the highest dose. Considering all these together it can be said that mitochondrial toxicity may be playing a role in cardiotoxicity induced by TK inhibitors.

**2067 The Cardiovascular Effects of Crotonaldehyde In Vivo and In Vitro**

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Crotonaldehyde (CR) is a highly electrophilic α,β-unsaturated aldehyde product of incomplete combustion in tobacco smoke and fossil fuels and of 1,3-butadiene metabolism. Little is known about cardiovascular effects of CR exposure. For an in vivo study, mice were exposed to CR gas (1 ppm, 6h/day) for 4 days (acute) or 12 weeks (chronic; 5 days/week). Telemetry was used to measure blood pressure and heart rate in acute CR exposure, while non-invasive blood pressure was measured by tail cuff once a week in a chronic study. Following CR exposures, aortas were isolated and vascular function was measured. Acute exposure to CR, heart rate decreased (63 bpm to 512 bpm; n=1) with no change in blood pressure. In contrast, blood pressure was significantly lower in CR group than in air controls in the chronic study. In isolated aorta of 4-day CR-exposed mice, the relaxation of aorta to sodium nitroprusside (SNP; an NO donor) was increased significantly and a similar response to acetylcholine (ACh) was observed relative to air control. Aortas of chronic CR-exposed mice had a similar response to SNP and ACh. In addition, sensitivity to phenylephrine (PE) was significantly reduced in aorta of CR-exposed mice. Because CR enhanced vascular sensitivity to NO (more vasodilation and lower blood pressure), we tested for a direct effect of CR on blood vessels, aorta and superior mesenteric artery (SMA) in vitro. CR induced relaxation in isolated aorta and SMA. SMA (EC50 = 6.1 µM) was more sensitive than aorta (EC50 = 72±7 µM) to CR. The concentration-dependent (1-300 µM) relaxation was inhibited significantly in SMA by the presence of: 1) mechanically-impaired endothelium; 2) Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME); 3) the calcium entry antagonist (A967079); and 4) a guanylyl cyclase (GC) inhibitor (ODO). Positive immunofluorescent staining for TRPA1 was co-localized in the endothelium of isolated aorta and SMA. In acute exposure, the inhibition of repolarizing K+ currents which has been observed in myocytes from marine species. Similar to marine species, this evidence presented here suggest that Phe is cardiotoxic to freshwater salmonids.
2068 Effects of Tobacco Product-Derived Unsaturated Aldehydes on Circulating Angiogenic Cells

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Tobacco smoking is the most significant modifiable risk factor in the development of cardiovascular disease. Mainstream cigarette smoke (MCS) has been linked to the development of systemic inflammation and endothelial dysfunction; endothelial function is a balance of injury and repair, the latter, in part, attributed to number/function of circulating angiogenic cells (CACs), wherein CAC blood levels are associated with endothelial health. Aldehydes are abundant toxins in tobacco-product aerosols, and previously, we linked acrolein exposure with cardiovascular toxicity in humans and mice; however, the effects of coirondehyde (CR), a similar short, unsaturated aldehyde, on endothelial repair are unknown. Thus, we performed a series of exposure studies (1 hpm; 4 days or 12 weeks, 5 days/week; 6 h/day) to determine the systemic and endothelium-related effects of CR exposure. Complete blood counts and plasma biomarkers were measured as indicators of systemic toxicity. The levels of CACs, specifically Flk-1+ Sca-1+ and Sca-1+ cells, were measured by flow cytometry. No significant changes were seen in WBC, RBC, or platelet counts after acute and chronic CR exposures. However, acute exposure decreased triglycerides levels, while chronic exposure increased levels of cholesterol (11±5%). High-density lipoprotein (16±7%), triglycerides (25±12%), and albumin (6±2%), although no changes were seen in Flk-1+ Sca-1+ cells after either exposure, were reduced by 4 days of exposure. But not after chronic exposure. Oxidative stress, as measured using monochlorobimane bound to glutathione, was unchanged in CACs or lymphocytes after exposure. Although CR and acrolein are similar, their effects after acute and chronic inhalation studies (1 ppm) were different. For example, acrolein significantly decreased CACs after 4 days and 12 weeks of exposure, whereas CR exposure did not. Contrary to these data, 4 days of MCS suppressed leukocytes and decreased Sca-1+ cells, but had no effect on Flk-1+Sca-1+ cells. Moreover, 12 weeks of MCS exposure decreased Flk-1+Sca-1+ cells, but had no effect on plasma lipids. Collectively, data from CR and acrolein exposure indicates that unsaturated aldehydes can affect different targets, yet their effects likely contribute to overall MCS-induced cardiovascular toxicity in complex ways. Future studies will define the mechanisms by which aldehydes as harmful and potentially harmful constituents of tobacco-based products induce cardiovascular toxicity.

2069 Gestational Inhalation Exposure to Titanium Dioxide Nanoparticles Increases Maternal-Fetal Vascular Resistance


The reproductive and cardiovascular effects of ENM exposure have been observed in adult populations, but the fetal consequences of gestational ENM exposure have yet to be fully understood. The placenta is a critical barrier protecting the fetus and allowing the transfer of nutrients from the maternal circulation. The purpose of this study was to determine the effects of pulmonary titanium dioxide nanoparticle (nano-TiO2) exposure on placental function and on the reactivity of the umbilical vasculature. We hypothesized that pulmonary nano-TiO2 exposure disrupts placental function and impairs umbilical vascular responsiveness. Pregnant Sprague-Dawley rats were exposed via whole-body inhalation to nano-TiO2, with a primary particle size of 21 nm on GD 11 for 7 days for a daily calculated lung deposition of 31±1 μg. Placentas, umbilical artery and vein were isolated, cannulated on GD 21 nm on GD 11 for 7 days for a daily calculated lung deposition of 31 ± 1.1 μg. Exposed rats of 62.58±4.7 mm Hg and 29.42±9.5 mm Hg respectively. Umbilical arteries from exposed dams had a decreased endothelium-dependent dilation (30.21±8.8%) compared to controls (57.92±6.1%) and an increased sensitivity to ANGII (−36.42±9.7%). These results suggest that gestational nano-TiO2 exposure impairs placental function and umbilical vascular reactivity in maternal-fetal vascular resistance. NIH R01-ES015022 (TRN), NSF-1003907(TRN, ABA), U54GM104942-02 (SH).

2070 Influence of Maternal Engineered Nanomaterial Inhalation on Uterine Adrenergic and Myogenic Microvascular Responses

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Engineered nanomaterials (ENM) are ubiquitously present in diverse human applications but have adverse cardiovascular effects. The reproductive and vascular consequences associated with ENM inhalation are not well understood. Myogenic reactivity is an adaptive gestational component that if impaired may have adverse effects on the maternal-fetal circulation. The adrenergic agonist norepinephrine (NE) exerts arteriolar vasococontractor effects and augments myogenic responsiveness. This study assessed uterine microvascular reactivity to adrenergic stimulation and transmural pressure changes after maternal nano-titanium dioxide (nano-TiO2) inhalation. Sprague-Dawley rats were exposed to nano-TiO2 aerosols during gestation. Evonik-P25 was utilized with a primary particle size of 21 nm and a zeta potential of -56.6 mV. Whole-body exposure (concentration = 10±0.5 mg/m3) was performed for 6 hours/d for 6 days (total lung burden = 217 μg). Rats were euthanized on day (GD) 20. Uterine microvascular reactivity was assessed via pressure myography. Experimental group characteristics: age 106±7 d, mean arterial pressure (MAP) 74±3 mm Hg, mass 372±28 g, litter size 14±1 pups, dry pup mass 0.49±0.02 g, dry placental mass 0.12±0.03 g. Sham characteristics: age 88±3 d, MAP 67±9 mm Hg, mass 387±10 g, litter size 13±1 pups, dry pup mass 0.66±0.09 g, dry placental mass 0.16±0.05 g. To stimulate only α-adrenergic receptors, β-receptors were inhibited with the α-antagonist propranolol (3X10-5 M). NE (1X10-5 M) was then applied. Myogenic responsiveness was assessed by pressurizing the vessels from 0-105 in 15 mm Hg increments. Subsequently, adrenergic facilitation of the myogenic response was evaluated by repeating the myogenic stimulus in the presence of NE. Myogenic reactivity was not significantly altered by ENM exposure (diameter change of 3.4±2.1%). Similarly, the adrenergic stimulation did not alter the myogenic response (4.7±1.7%) nor was the percent maximal response to NE at 60 mm Hg (3.1±1.0%). These results are preliminary ongoing experiments. Additional experimental groups and vasoactive agonists are being assessed (angiotensin II). Support: E0515022 (TRN), U54GM104942 (SH).

2071 Association of Exposure to Volatile Organic Compounds and Cateholamines


Stress is a major risk factor for the development of cardiovascular disease. Human response to stress occurs through activation of the sympathetic nervous system (SNS) to induce a fight or flight response. SNS activation in turn elicits a release of stress hormones like catecholamines. Exposure to toxic environmental chemicals has been shown to induce and stress, thus triggering a response of the SNS. We hypothesized that exposure to volatile organic compounds triggers an SNS response and subsequent release of catecholamines. Our cross-sectional study consisted of 260 nonsmokers who had low to high cardiovascular disease risk. On the day of enrollment, blood pressure of the participants was measured and urine samples were obtained. Urinary levels of ten catecholamines and metabolites and twenty parent VOCs (26 metabolites) were quantified by ultra fast performance liquid chromatography-mass spectrometry. Generalized linear models were used to examine the association between VOC metabolites and catecholamine metabolites in urine. We found significant associations with the two major catecholamines norepinephrine and epinephrine. Norepinephrine was significantly associated with the styrene metabolite, mandelic acid (p=0.0015). Epinephrine was significantly associated with metabolites of coirondehyde, N-acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine, and 1,3-butadiene, N-acetyl-S-(4-hydroxy-2-buten-1-yl)-L-cysteine (p=0.0361 and p=0.0258, respectively). Vanillylmandelic acid is the parent compound of epinephrine and norepinephrine. Vanillylmandelic acid was significantly associated with the styrene metabolite, mandelic acid, and toluene metabolite, N-acetyl-S-(benzyl)-L-cysteine (p=0.0007 and p=0.0008). Exposure to environmental chemicals such as volatile organic compounds stress the human response and may contribute to the development of cardiovascular disease.
Safety-related attrition remains a major issue in drug discovery and development. To address this problem, the CIPA paradigm is being integrated into phenotypic characterization of targeted agents. The role of MyD88 signaling in the intestinal cell cycle progression and explains the lack of GI toxicity observed with palbociclib.

In the current study, the IEC6 cell line, a non-transformed rat small intestinal epithelial cell line with well-defined mouse model of nonischemic HF induced by Transverse Aortic Constriction (TAC), we previously demonstrated that Type 1 helper CD4+ (Th1) cells migrate to the heart and induce adverse cardiac remodeling. Moreover, T cell deficient (tcrα−/−) mice are protected from HF, while adoptive transfer of wt Th1 cells to these mice reconstitutes the pathology of HF. The adapter protein myeloid differentiation primary-response protein 88 (MyD88) is an important proinflammatory signaling molecule in innate leukocytes; and recently has been shown to be important in adaptive CD4+ T cells as well. Currently, we demonstrated that adoptive transfer of myD88+ Th1 cells to myD88−/−tcrα−/−host mice results in accelerated progression of HF with significantly higher numbers of Th1 cells in the heart. Therefore, we hypothesize that MyD88 regulates survival and proliferation of Th1 cells, and modulates the pathology of nonischemic HF. To address this, we generated in vitro differentiated myD88−/− and wt Th1 cells to evaluate differential survival and proliferation. Using a combination of flow cytometry and Cytation cell imaging, we demonstrate that myD88− Th1 cells have significantly enhanced survival and proliferation in comparison to wt Th1 cells. These data reveal a new pathway in Th1 cells that control cell survival, and hence the magnitude and duration of inflammation in vivo.

Electronic cigarettes (e-cigarettes) are popular nicotine delivery devices first introduced to the United States in 2007. Despite being advertised as a healthier alternative to conventional tobacco, the health consequences of e-cigarette use remain under investigated. The wide variety of flavors and e-cigarette models makes e-cigarettes appealing to adult and youth smokers and never-smokers. Their growing popularity combined with possible health consequences suggest a need to further research potential health hazards of electronic liquids (e-liquids) used in e-cigarettes. Since conventional tobacco use is a risk factor for osteoporosis, this study examines whether exposure to e-liquids affects bone-forming osteoblasts. Our previous research indicates e-liquid osteotoxicity is flavor-dependent, with flavorless e-liquids being the least osteotoxic and cinnamon-flavored e-liquids being the most osteotoxic. Hence, this study focuses on flavorless and cinnamon-flavored e-liquids. Human osteoblast-like MG-63 cells were exposed for 48 hours to 0.04%, 0.4%, 2% or 4% of unvaped nicotine-free e-liquids or 0.0025%, 0.025%, 0.25% or 2.5% of vaped nicotine-free e-liquids or to a culture medium only control. Changes in cell viability were assessed by MTT assays, and the expression of a key bone protein, collagen type I, was analyzed by immunofluorescence. Cell viability decreased in a dose-dependent manner, which was most pronounced with cinnamon-flavored e-liquids whether the e-liquid was unvaped or vaped. There were no detectable changes in collagen type I protein following exposure to any of the vaped e-liquids. This study demonstrates that osteoblast-like cells are sensitive to both unvaped and vaped e-liquids, particularly to the cinnamon-flavored ones. Additionally, collagen type I does not appear to be a target for the osteotoxicity of vaped e-liquids. In order to understand the mechanism behind e-liquids osteotoxicity, ongoing studies are investigating the effects of e-liquids on oxidative stress. This study provides insight into the potential impacts of e-cigarette 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 Number P20GM103408.
2077 Cytotoxic Mechanism of a Mitomycin C-Lexitropsin Hybrid Anticancer Drug

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Hybrid anticancer drugs have drawn lots of attention for cancer research to improve clinical efficacy and to target various types of cancer. Mitomycin C and lexitropsin are potent anti-cancer agents, but with different DNA interaction mechanisms. The hybrid compound obtained from linking the pyrrole fragment backbone of lexitropsin to a mitomycin moiety was investigated for both cytotoxic activities against breast cancer cells and molecular pharmacological mechanism. The activation reactions were studied via theoretical (quantum chemistry) calculations. These calculations showed that the mitomycin moiety linked to one pyrrole fragment causes a more favorable energy of reduction than mitomycin moiety linked to two pyrrole fragments or mitomycin alone. Cytotoxicity results indicate that the former is much more effective on killing both breast cancer cells used in this study, MCF-7, and MDA-MB 468 cells. Both compounds have a stronger cytotoxic effect on MDA-MB-468 cells, which are triple negative breast cancer cells with p53 mutation. Mitomycin with one pyrrole ring also can effectively inhibit microtubule polymerization and trigger DNA crosslink at different temperatures. The results from theoretical activation energy calculations and cytotoxic analysis suggested that the compound with one pyrrole ring attached to mitomycin was much more effective on triggering breast cancer cell death. Moreover, this hybrid compound was able to destroy microskeletal network and also able to trigger DNA damage.

2078 Methyglyoxal-Induced Advanced Glycation End Products Promote Proliferation via MAPK/P38/Akt Signaling Pathways in Renal Cell Carcinoma Cell

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Sponsor: J. Lee

Advanced glycation end products (AGEs) are the final products formed when carbohydrate-rich food is heated at high temperatures. Although the underlying mechanism of neurotoxicity still remains elusive. Our previous studies showed that the level of noradrenaline declined, and the density of noradrenergic nerve fibers decreased in the brains of rats exposed to ACR by gavage, and that LC3B positive cells were observed in the murine cerebellum. The present study aims to investigate the cytotoxicity of acrylamide in neuron and microglial cell. Firstly, mouse noradrenergic neuron CATH.a and mouse microglial cell BV2 were exposed to acrylamide for 24 hours at different concentrations. Cell viability, LDH, and caspase 3/7 activity were examined as described above. In CATH.a neuron, while the cell viability decreased on the ACR concentrations, the quantity of LDH and caspase 3/7 activity remained unchanged. However, with chloroquine, not LC3B positive cells were observed in the murine cerebellum. The present study aims to investigate the cytotoxicity of acrylamide in neuron and microglial cell. Firstly, mouse noradrenergic neuron CATH.a and mouse microglial cell BV2 were exposed to acrylamide for 24 hours at different concentrations. The present study aims to investigate the cytotoxicity of acrylamide in neuron and microglial cell. Firstly, mouse noradrenergic neuron CATH.a and mouse microglial cell BV2 were exposed to acrylamide for 24 hours at different concentrations. The present study aims to investigate the cytotoxicity of acrylamide in neuron and microglial cell. Firstly, mouse noradrenergic neuron CATH.a and mouse microglial cell BV2 were exposed to acrylamide for 24 hours at different concentrations. The present study aims to investigate the cytotoxicity of acrylamide in neuron and microglial cell. Firstly, mouse noradrenergic neuron CATH.a and mouse microglial cell BV2 were exposed to acrylamide for 24 hours at different concentrations. The present study aims to investigate the cytotoxicity of acrylamide in neuron and microglial cell. Firstly, mouse noradrenergic neuron CATH.a and mouse microglial cell BV2 were exposed to acrylamide for 24 hours at different concentrations. The present study aims to investigate the cytotoxicity of acrylamide in neuron and microglial cell. Firstly, mouse noradrenergic neuron CATH.a and mouse microglial cell BV2 were exposed to acrylamide for 24 hours at different concentrations. The present study aims to investigate the cytotoxicity of acrylamide in neuron and microglial cell. Firstly, mouse noradrenergic neuron CATH.a and mouse microglial cell BV2 were exposed to acrylamide for 24 hours at different concentrations.
2081 Integrated Networking Analysis of Toxicological Mechanisms of Natural Compounds Using Bio-Sensing Cell Lines


Natural products have been developed as therapeutic agents for various diseases including cancer in the drug discovery area. However, the potential toxicity of natural products could be a big hurdle to develop the drug candidates. To overcome this limitation, the development of high-contents screening method to give the information about toxic mechanism is needed. Here, we suggest the biosensing method to evaluate the cellular stress for the natural compounds using bio-sensing analysis. Several transcriptional factors including AP-1, P53, Nrf2, and NFkB are regulated during an early toxic response by triggering cell proliferation, apoptosis, oxidative stress, and inflammation. To screen the cellular stress response, we constructed the established HepG2 cell lines to express the luminescence and GFP when only these transcriptional factors are activated using the transcription response element. Using biosensing hepatocytes, we screened the toxic effects for the wide ranges of twenty natural compounds including flavonoid, poly cyclic, di-terp-period, and quinoid and quantitative changes were examined by imaging analysis. Several natural compounds including apigenin, the potential hepatotoxic compounds cause the cytotoxic effect mediated by AP-1, and P53 pathways and we can classify the mode of action for the natural compounds using this system. Our results suggest that the biosensing hepatocytes are suitable for an early sensing method for screening the hepatotoxicity and could give critical information about molecular mechanisms to initiate the cellular stress. This approach can be used to build up the adverse outcome pathways (AOP) to unravel the key events associated with liver injury for natural compounds. This work was supported by a grant (NRF-2016M3A9C4953144) from the Ministry of Science, ICT, and Future Planning and a general research grant from the Korea Institute of Toxicology.

2082 Trovafloxacin-Induced Protective Autophagy Is Inhibited by TNFalpha-Mediated Upregulation of mTOR Pathway in HepG2


Hepatotoxic drugs synergistically increase liver toxicity by specific cytokines. In this study, we describe that alteration of autophagy plays an important role in the synergistic effect of drug-induced immune-mediated hepatic injury in human hepatoma cells. We found that TNFα increases the Trovafloxacin-induced cytotoxicity in HepG2 cells but does not affect Levofoxacin- and Nefazodone-induced cytotoxicity. In addition, the Trovafloxacin-induced autophagy is dependent on the mTOR signaling pathway and is the result of the proteasomal degradation of mTOR protein and downstream-proteins. The autophagy-inhibited ATG5 knockdown HepG2 cells increase apoptosis signal, and cytotoxicity by Trovafloxacin compared to the untreated controls and these results suggest that Trovafloxacin-induced autophagy plays a protective role. We found that the treatment of Trovafloxacin up-regulates the phosphorylation of p70S6K(T389) among the mTOR signals, thereby inhibiting Trovafloxacin-induced protective autophagy, which suggests that TNFα that can increase hepatotoxicity through the collapse of the autophagy mechanism by Trovafloxacin. Taken together, our study suggests that autophagy may be the causative factor of idiopathic hepatotoxicity including inflammatory mediators of hepatotoxicity, and may be used as a new parameter to predict toxicity through identification of autophagy mechanisms. This work was supported by a grant (NRF-2016M3A9C4953144) from the Ministry of Science, ICT, and Future Planning and a general research grant from the Korea Institute of Toxicology.

2083 Characterization and Application of a 3D Human Oral Buccal Model for Whole Cigarette Smoke and Smokeless Tobacco Exposure

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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. However, epidemiology shows that the risk of oral cancer is far higher for smoking than for smokeless tobacco use. The goals of this study were to 1) assess the consistency of whole cigarette smoke-induced cytotoxicity in a 3D human oral buccal model, EpiOral™, using tissues obtained from two donors and 2) to further characterize cytotoxic and oxidative stress responses following exposure to extracts of CORESTA smokeless tobacco reference products (CRPs) in this model. Whole smoke exposures were conducted by exposing the apical side of EpiOral™ tissues to either 3RF4 whole smoke (Health Canada Intense smoking regimen, 0.5 L/min dilution airflow, 20 mL/min vacuum) generated by a VITROCELL® VC1 smoking robot or concurrent clean air control up to 2 hours. Cytotoxicity by either 3-(4,5-dimethyl-2-y1)-2,5-diphenyloxazolium bromide (MTT) or lactate dehydrogenase (LDH) assays was measured 24 hours post exposure. CRPs for snus (CRP1), moist snuff (CRP2), and dry snuff (CRP3) were each exposed 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 applied to the apical side of EpiOral™ tissues for 48 hours continuously and cytotoxicity (MTT/LDH) and oxidative stress (8-iso-prostanet) were measured. A consistent dose-dependent response with significant cytotoxicity was observed in both donor tissues following exposure to whole smoke. IC50 values (mean ± SD) for MTT were 12.64 ± 0.51 cigarettes and 13.69 ± 0.83 cigarettes for donors A and B, respectively. In contrast, minimal (< 20%) cytotoxicity was observed in tissues exposed to CRPs. While the CRP extracts elicited minimal cytotoxicity, dose-dependent effects on oxidative stress were observed: the release of 8-iso-prostanet showed significant increases for each reference product for the two donors; however, the response between products was significantly differentially induced. Collectively, the data suggest that the 3D human oral buccal model, EpiOral™, may be useful in differentiating between tobacco product categories.

2084 Differential Toxicity, Uptake, and Release of Inorganic Mercury and Methyl Mercury in Human Endothelial Cells

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Cardiovascular disease (CVD) is the leading cause of morbidity, mortality, and health care costs in the United States, and around the world. Among the various risk factors of CVD, environmental and dietary mercury (Hg), a highly toxic metal traditionally labeled as a neurotoxin, is a potential contributor towards human atherosclerotic development. In this study, we investigated the toxicity, type of cell death, dose-dependent uptake, and release of two forms of Hg commonly exposed in the environment, i.e., inorganic HgII (as HgCl2) and methylmercury or MeHg (as CH3HgCl) in EA.hy 926 endothelial cells. Our results showed that HgII is highly toxic to the endothelial cells, owing to the higher uptake into the cytoplasm and perhaps more importantly less HgII is released than MeHg by the cells, thus the “net” accumulation by the endothelial cells is higher for HgII than MeHg when exposed to the same Hg levels. Exposure to MeHg also increased IL-8 gene expression dramatically. Furthermore both HgII and MeHg were found to induce apoptotic and necrotic cell death. This study has important implications to further understanding of the toxicity of these two common Hg species (obtained upon exposures to different environmental and dietary media), and their contributions to the development of atherosclerosis, a process leading to CVD.

2085 Diphenyl Diselenide Induces Cytochrome C-Dependent Apoptotic Pathway in Triple Negative Breast Cancer Cells


Triple-negative breast cancer (TNBC) is one of the most common and aggressive types of breast cancer. TNBC cells lack the expression of three key receptors: progesterone, estrogen and HER2, hence cannot be treated using existing targeted therapies. Therefore TNBC patients have an extremely poor prognosis compared to other sub-types of breast cancer. Previous studies have shown that selenium containing drugs can treat a range of disorders. In particular diphenyl diselenide (PDPS) has been reported to be cytotoxic against cancer cell lines at concentrations greater than 30 µM. However the effect of PDPS on breast cancer has not been studied. Here we investigated the effect of two synthetic organoselenium derivatives, DPDS and diphenyl selenide (DPS) on three TNBC cell lines (MDA-MB-231, MDA-MB-468 and Hs 578T), and compared drug action with estrogen positive breast cancer cells (MCF-7), human epidemial growth factor receptor 2 positive breast cancer cells (SKBR3), and two non-cancerous cell lines (MCF-10A and human normal dermal fibroblasts). Initially, cell death induced by DPS and DPDS was determined by MTT and Hoechst/PI double labeling assays. DPS showed no anti-cancer effect against breast cancer or non-cancerous cell line; but DPDS showed potent cytotoxicity, with IC50’s in the range 7-18 µM against TNBC cells, and 27 µM against MCF-7s. Interestingly however DPDS did not display cytotoxicity towards SKBR3 cells, or against any of the non-cancerous cell lines. This approach can be used to build up the adverse outcome pathways (AOP) to unravel the key events associated with liver injury for natural compounds. This work was supported by a grant (NRF-2016M3A9C4953144) from the Ministry of Science, ICT, and Future Planning and a general research grant from the Korea Institute of Toxicology.
cells, suggesting selectivity towards TNBC. The data also indicated that DPDS displayed higher cytotoxicity towards MDA-MB-231 cells compared to the other breast cancer cell lines. Therefore we performed further studies with MDA-MB-231 cells to determine the mechanism of DPDS induced cell death. The TO-PRO-1 probe was next used to identify if DPDS caused apoptosis. Quantitative analysis indicated that after 18 hours DPDS had initiated cell death via apoptosis in 20-25 % of the cell population. Next, the expression of cytochrome c from the mitochondria in DPDS treated apoptotic cells was determined by immunocytochemistry. Fluorescence micrographs identified that 20-30 % of mitochondrial cytochrome c was released after only two hours treatment with DPDS, leading to the rapid onset of apoptosis. This was accompanied by a two-fold increase in the activity of caspase-3, detected using the Ac-DEVD-AMC caspase-3 fluorogenic substrate. These data suggest that DPDS is a potential anti-cancer drug capable of selectively activating the intrinsic pathway of cell death in TNBC cells.

**2086 Perhexiline Induces Mitochondrial Dysfunction and Apoptosis in HepG2 Cells**


Perhexiline is a prophylactic antianginal agent developed in the 1970s. Despite its success, its use diminished due to the occurrence of poorly understood side effects including severe hepatotoxicity in certain patients. Here we present the results of studies examining whether or not hepatocyte injury is associated with mitochondrial dysfunction and programmed cell death in both HepG2 cells and HepARG cells. Particularly, the decline of cellular ATP levels caused by perhexiline was dramatically exacerbated when galactose was substituted for glucose as the sugar source, suggesting a potential mitochondrial liability. JC-1 staining confirmed the occurrence of mitochondrial membrane depolarization upon treatment of perhexiline. Bongkrekic acid, a mitochondrial permeability transition pore blocker that targets adenine nucleotide translocase, attenuated both the ATP depletion and LDH leakage induced by perhexiline. Perhexiline significantly increased the activity of caspase 3/7 and caspase 9, and also elevated the gene expression of TNFα on the mRNA level, suggesting that perhexiline activated apoptosis in HepG2 cells. Pretreating cells with pan caspase inhibitor Z-VAD-FMK, or with inhibitors specific for caspase-3, -8, and -9 respectively, attenuated the cytotoxicity of perhexiline. Interestingly, treating HepG2 cells with NecroX S, a necrosis inhibitor, also partially protected cells from the toxicity of perhexiline, suggesting that necrosis may play a role in the cytotoxicity of perhexiline. In addition, the expression levels of Bcl-2 family proteins and TNFα showed significant changes upon perhexiline treatment. Overall, our results suggest that perhexiline induced mitochondrial dysfunction and apoptosis in HepG2 cells.

**2087 The Molecular Mechanism of Cigarette Smoke Induced Necroptosis on Human Vascular Endothelial Cell**

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Cigarette smoking is a well-established risk-factor for cardiovascular disease and a major cause for atherosclerosis. Some chemicals in cigarette smoke can activate reactive oxygen species (ROS) generation and disrupt the microenvironment in which the vascular endothelial cells (VEC) reside, inducing the death of endothelial cells as well as a series of pathological responses. It is well known that ROS can trigger endothelial cells apoptosis in the animal models and human samples. However, it remains unclear whether VEC necroptosis occurs when ROS accumulate and what its underlying mechanism is. In our preliminary experiment, we investigated the effect of the cigarette smoke extracts (CSE) on the vascular endothelial cells. Utilizing time-lapse microscopy, we found there were cell apoptosis and necroptosis simultaneously, which initiated after cigarette smoke extracts exposure 3h and gradually increased after exposure 6h. The FACS also revealed that there were obvious cell apoptosis and necroptosis after exposure 6h, and the most of cells were necrosis at 24h. Meanwhile, we observed ROS generation significantly increased in the HUVECs at exposure 3h and 24h respectively. As RIP-RIP3-MLKL pathway played key roles in the cell necroptosis, we further tested their expression level by western blotting. The CSE obviously induced phosphorylation of RIP3 and MLKL at exposure at 24h. Next, we treated HUVECs with N-Acetyl-L-cysteine (NAC), which is commonly used to inhibit ROS generation. The CSE-induced ROS generation was dramatically inhibited by NAC pretreatment, and necroptosis was partially inhibited by NAC-1 as well. Moreover, NAC significantly inhibited RIP3 and its phosphorylation level. These results demonstrated that ROS-induced necroptosis through regulation of RIP3 and its phosphorylation. These studies of the regulatory mechanism and related pathways could help us better understand the pathogenesis of smoking induced arteriosclerotic vascular disease and provide a potential avenue for prevention and treatment.

**2088 Cadmium Exposure Induces Pancreatic β-cell Dysfunction and Death via a Ca²⁺-Triggered JNK/CHOP-Related Apoptotic Signaling Pathway**

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Cadmium (Cd) is known to be ranked the 7th hazardous substance in the Substance Priority List by Agency for Toxic Substances and Disease Registry. The experimental and epidemiological data have indicated that Cd is linked to the development of diabetes mellitus. The molecular mechanism of Cd on pancreatic β-cell cytotoxicity still remains unclear. Evidence has pointed toward that Ca²⁺ is an important regulator of toxic insult-induced β-cell cytotoxicity. The role of Ca²⁺ in the Cd-induced β-cell cytotoxicity is still unknown. In this study, we found that Cd exposure significantly inhibited insulin secretion and cell viability in the pancreatic β-cell-derived RIN-m5F cells. Cd exposure increased the populations of apoptotic cells and sub-G1 hypodiploid cells. Cd exposure induced apoptotic signaling caspase-3/-7/-9 activation, which largely depended on the activation of c-Jun N-terminal kinase (JNK) and C/EBP homologous protein (CHOP). Transfection with siRNAs for JNK and CHOP or pretreatment with specific pharmacological inhibitor of JNK (SP600125) in β-cells effectively prevented the Cd-induced insulin secretion dysfunction and apoptosis. JNK-specific siRNA dramatically suppressed Cd-induced JNK phosphorylation and CHOP protein expression, but JNK phosphorylation could not be inhibited by CHOP-specific siRNA. Furthermore, Cd exposure significantly increased the intracellular (Ca²⁺)⁰ levels. Buffering the Ca²⁺ response with BAPTA/AM effectively abrogated the Cd-induced [Ca²⁺]⁰ elevation, insulin secretion dysfunction, apoptosis, and protein expression of phosphorylated JNK and CHOP. Taken together, these findings demonstrated that Cd-induced β-cell dysfunction activates the extracellular Ca²⁺ entry via a [Ca²⁺]⁰-dependent JNK activation-activated downstream CHOP-related apoptotic signaling pathway.

**2089 Benchmark Dose (BMD) Modeling of Image-Based Phenotypic Profiling Data Yields More Potent Estimates of Chemical Bioactivity Compared to Cell Viability and Apoptosis Assays**


High-throughput imaging-based phenotypic profiling (HTPP) is a chemical screening method that combines automated microscopy and image analysis to identify a large variety of morphological features at the single cell level. Here we describe workflows for concentration-response screening and image analysis using an HTPP assay that quantitatively evaluates changes in organelle morphology (i.e. Cell Painting), as well as calculation of biological pathway altering concentrations (BPACs) using high-throughput concentration-response modeling (BMDExpress 2.2). A set of 16 reference chemicals were tested in six human cell lines (U-2 OS, MCF7, HTP-9, A549, ARPE-19, HepG2). Cells were plated in 384-Well plates and after 24 h treated with 7 concentrations (semi-log spacing, n = 3/plate, 3 cultures) in a randomized pattern. After 48 h, cells were live labeled with MitoTracker (mitochondria), fixed, permeabilized and labeled with Hoechst-33342 (nuclei), SYTO14 (nucleoli) and fluorescent conjugates of concanavalin A (ER), phallolidin (cytoskeleton), and wheat germ agglutinin (Golg/plasma membrane). A multiplexed cell viability (CV) and apoptosis (AP) assay was run in parallel. Confocal images were acquired using an Opera Phenix HCS system and analyzed using Harmony software, yielding ~1200 features per cell. Cell-level data were median absolute deviation (MAD) normalized to DMGO controls. BMD modeling was performed on well-level median values. Most chemicals (n=14) affected cell morphology in a concentration-dependent manner. Distinct patterns of affected cellular features were observed across the chemical set and, in most cases, were consistent with observations from the literature. In general, the chemically most potent cell patterns, with high-confidence toxicity estimates, were across the different six cell lines. For all compounds, HTPP BMDs were at least as sensitive as CV or AP BMDs. In some cell lines, profiling BMDs were >10x lower than CV or AP BMDs. Screening of a larger set of chemicals (n=480) also
A Unique Comparison of Three Intrathecal Administration Techniques for Non-Systemic Delivery of CNS-Targeting Antisense Oligonucleotides in Cynomolgus Monkeys for the Benefit of the 3Rs

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Single oligonucleotides have been reported to deliver 5-10x higher brain exposure levels with implanted port catheter (PC) administration than with lumbar puncture (LP). Hence, we designed a study in NHP to compare exposure in the CNS with different IT administration methods with the same LNA (locked nucleic acid) dose and volume. Six Mauritius male monkeys (3.0-3.5 kg and 2 to 5 years) underwent one of the following intrathecal dosing regimens, followed by 1.5 mL artificial cerebrospinal fluid (CSF) (two animals/group): 1) Standard intrathecal bolus administration with a pediatric non-coring needle (LP, lumbar puncture); 2) Intrathecal implanted port catheter (PC, catheter tip reached T10 through T12) and 3) Temporary intrathecal catheter (TC, catheter tip reached T10 through T12). Bioanalysis was conducted on plasma, CSF and on neuronal tissue collected at necropsy 15 days after dosing. Brain exposure using LP administration was up to 2x higher than using PC administration. An increased volume of administration and flushing appears to be the most significant factor to achieve higher brain concentrations with a single administration schedule since previous studies with lower volumes resulted in 10-20x lower exposure using LP administration. Exposure in liver was also about 2x higher with LP. In terms of histopathology, only the well documented procedure-related findings at the injection/implantation sites were observed. In summary, the conducted lumbar bolus method may reduce the number of similar studies in the future, potentially reducing animal usage (as no biocytological comparison study is required) and surge 2, as it was confirmed that a specialized lumbar administration technique, using non-coring pediatric needles, does show similar pharmacokinetic exposure levels.

Two Cases of Lumbar Spinal Cord Infarction in Cynomolgus Monkeys Associated with Intrathecal Bolus Administration

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Many potential drugs against neurodegenerative diseases, particularly antisense oligonucleotides (ASO), are administered via intrathecal injection, as they do not cross the blood-brain barrier. This route of administration is well established in cynomolgus monkeys that are used for preclinical safety assessment when other species cannot be used. We have conducted intrathecal injections in >1800 individual monkeys and among those we have observed two cases of spinal cord infarction. This poster describes clinical symptoms, diagnostic approaches, morphological features, and prognosis of this rare injury. Both animals (males) were intrathecally (lumbar) administered an ASO under standard conditions (anesthesia, withdrawal of equivalent CSF volume ahead of dosing, manual bolus infusion over 1 minute using a pediatric pen cil-point needle). They showed lameness of both hind limbs and absence of patellar and foot grip reflexes approximately 0.5 and 4 hours after dosing (case 1 and 2, respectively). Approximately 240 and 96 hours after the administration, respectively, Magnetic Resonance Imaging (MRI) of the lumbar spine was performed under anesthesia employing a 1.5 Tesla MRI scanner (GE Healthcare). In both cases, MRI scanning revealed a linear hyper-intense signal of the lumbar spinal cord, compatible with a spinal cord infarct. In both cases, the locomotor impairment was reversible within 3 weeks. In a follow-up MRI scan of case 1 9 weeks later, the area of hyper-intensity was still present and larger in size (2.8 mm antero-posterior, 3.5 mm latero-lateral and 40.0 mm crano-caudal compared to 2.4 mm, 3.4 mm and 17.0 mm, respectively, during the first scan). Necropsies were performed 29 and 27 days after the administration. Microscopic evaluation of the lumbar intrathecal injection site revealed necrosis within the spinal cord resulting in malacia of the dorsal portion of the cord in case 1 and focal axonal degeneration, foamy macrophages and multifocal vacuolation with axonal degeneration of spinal nerve roots in case 2. MRI represents a useful non-invasive diagnostic method to confirm spinal cord infarction, a very rare mechanical, intrathecal dosing-related complication in cynomolgus macaques.

Default Occupational Exposure Limit for Single-Strand Oligonucleotides in Early Development

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Development of single strand oligonucleotides (SSOs) as potent pharmacueticals has experienced a boost over the last years. At the moment, there are more than 100 clinical studies ongoing (ClinicalTrials.gov). Therefore, occupationa l toxicologists are challenged by an increasing number of requests to set occupational exposure limits (OELs) for SSOs, especially early in development. Current generation SSOs therapeutics are oligomers of synthetic nucleotides typically of 12 to 20 units in length (MW typically in the range 5000-7000 Da) designed to hybridize to endogenous or pathogens’ nucleic acids according to Watson-Crick base pairing. To improve drug-like properties, their inter-nucleotide links are generally chemically modified, resulting in a diverse class of molecules. Despite their different backbones, common features have been described with regards to toxicological effects. This poster summarizes these similarities. In addition, a default OEL limit in the range of 10-100 µg/m³ has been derived for molecules in early development with limited substance specific data. The default value has been based on intensive review of toxicological and pharmacokinetic data as well as an assessment of currently set OELs for SSOs from 4 companies.

Safety Assessment of Ionis-MAPT mRNA Microtubule-Associated Protein-Tau (MAPT) Lowering Antisense Oligonucleotide (ASO), by Intrathecal (IT) Lumbar Puncture (LP) for 13 and 39 Weeks in Cynomolgus Monkeys

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IONIS-MAPTₚₜₜᵣᵣ is a 2’-O-methoxymethyl ASO targeting MAPT mRNA for the treatment of dementia associated with increased levels of tau protein in the spinal cord and brain. Centrally-acting ASOs need to be given IT as they do not cross the blood-brain barrier. Preclinical safety of IONIS-MAPTₚₜₜᵣ, which has pharmacologic activity in cynomolgus monkeys, was assessed in this species at three dose levels (4, 12, and 35 mg), as a once monthly repeat-dose IT LP bolus injection of 1.0 mL at lumbar (L) 3 - L4 for 13 or 39 weeks. No adverse test article-related effects on neurobehavioral, clinical pathology, CSF cell counts, ophthalmic examinations, electrocardiogram and blood pressure were recorded. There were also no organ weight changes or macroscopic findings related to treatment. Non-adverse, minimal focal neuronal vacuolation in hippocampus, a typical feature of central ASO administration, was an inconsistent finding across 13-week and 39-week studies. Fluorodacade staining of these hippocampal neurons in the 13-week study showed no fluorescence to indicate the presence of degeneration. Other IONIS-MAPTₚₜₜᵣ-related, non-adverse, microscopic observations were mononuclear cell inclusions (nervous system) and granular macrophages (lymph nodes) at all doses. Dose-dependent increase in exposure following IT administration was characterized by increasing concentrations of IONIS-MAPTₚₜₜᵣ in brain, spinal cord, kidney cortex and liver. In the CNS, exposure was generally greatest in the spinal cord with slightly lower levels in the brain. As expected, there was a significant decrease in MAPT mRNA and total tau protein in spinal cord and multiple CNS regions, as compared to controls. In conclusion, IT administration of 4, 12, or 35 mg of IONIS-MAPTₚₜₜᵣ in cynomolgus monkeys for 3 or 9 months (5 to 10 total doses) showed good local and systemic tolerability, no adverse neurobehavioral effects, and no adverse microscopic findings.
Gene therapy is a promising area of drug development for a number of diseases including some not treatable by approved medical treatments. Gene therapy works by delivering nucleotide sequence homology with humans, and need to be used when they are the only species where test article cross-reactivity occurs. An IACUC-approved regulatory toxicity study required bilateral administration of a gadolinium labeled viral vector into four locations into the Striatum with temporarily implanted catheters. Magnetic resonance imaging (MRI) was used for calculation of trajectory and for surveillance of test article delivery during convection-enhanced delivery. 24 monkeys were prepared for a 6-8 h anesthesia including pre-surgical analgesia, induction of anesthesia with ketamine / medetomidine, intubation and placement in a stereotactic frame. Induction anesthesia was conducted with isoflurane. Animals were transferred to the MRI to obtain data for calculation of position, angle and depth for catheter placement, assuring that the trajectory will not cross blood vessels or ventricles. Following transfer to the surgical suite, the stereotactic frame was used to ensure correct placement of the catheters based on the coordinates determined by MRI. The skin was reclined from the skull and holes were carefully drilled. Catheters were cut to specific length and inserted along the calculated trajectories to administer the viral vector to 4 different locations into the Striatum. Catheters were connected to infusion lines containing the gadolinium labeled test article. Infusion (rate 0.3 mL/h) was monitored in the MRI for 80 min to verify correct administration. Thereafter, animals were transferred back to the surgical suite and catheters were explanted. Animals recovered from the 6-8 h anesthesia within 15-30 min and were treated with analgesics and antibiotics. Animals presented laryngeal swelling and were treated with corticosteroids successfully. No animal showed neurological abnormalities. In conclusion, 96 catheters were successfully implanted in 24 animals, allowing intracranial administration into the Striatum (Caudate nucleus and Putamen). Six-week-old female Crl:CD1(ICR) mice were used. Blood sampling was performed 2 (at 0.5 and 4 h) or 3 times (at pre and 24 h) within 24 h from the tail vein by sparse sampling on Day 0. The following sampling volumes were selected: 2.2% (2 x 20 mL), 3.3% (2 x 30 mL), 3.3% (3 x 20 mL), and 5.0% of CBV (3 x 30 mL). After blood sampling, hematology was conducted on Days 1 and 3, and the animals were then necropsied and examined histopathologically. Clinical signs, body weight, and organ weights were also evaluated. Results: No abnormalities were noted in clinical signs, body weight or organ weights in any mouse. No abnormalities were noted in hematology at 2.2% or 3.3% of CBV. Sampling 5.0% caused low erythrocyte count (RBC), hemoglobin concentration (HGB), and hematocrit value (HCT) on Day 1, both reticulocyte ratio (RET) on Day 3. No histopathological abnormalities were observed at up to 5.0% of CBV. Discussion and Conclusion: Based on the above-mentioned results in mice, sampling 5% of CBV caused anemia and hemopoiesis. Low RBC, HGB and HCT on Day 1 and high RET on Day 3 were noted, although no histopathological abnormalities were observed. However, sampling approximately 3% of CBV did not cause any anemia or hemopoiesis. These results suggested that the relationship between total blood sampling to CBV ratio and anemia or hemopoiesis was almost the same in both mice and rats. Accordingly, we concluded that, in rodents, microsampling up to 3% of CBV can be used for both toxicological evaluation and confirmation of systemic exposure in the same animal.

In recent decades, despite dramatic increases in investment, pharmaceutical research and development productivity declines. A recent survey showed that most important reason for drug failure is off-target and on-target related safety, explaining more than 50% of all project closures. In order to help assess target safety we generated a comprehensive database and software. We made computable data from publicly available databases and internally curated data on proteins, biologically active chemicals, their interactions, pathways and pathologies. We cataloged >4 million references, supporting each database entry. All interactions are hyperlinked to appropriate PubMed article as support, while proteins and chemicals are hyperlinked to EntrezProtein, PubChem or the other appropriate public database. We developed toxicology ontology with >2500 toxicity endpoints to support data integration. The system can be queried with protein name or sequence, chemical structure or text searches for toxicities and pathologies. We tested the system by exploring androgen receptor (AR), a high data density target with known safety liabilities. Mapping AR onto GO function module within the system promptly identified AR biological function in sex differentiation and male gonad development including prostate gland development. Mapping AR together with its interacting proteins onto Diseases and Organ and tissue pathologies module, associated AR with several pathologies and toxicities related to development including Swyer syndrome and Y chromosome deletion. In addition, the system associated AR with other pathologies on cellular, organ and organ system level related to cardiac system (heart fibrosis, cardiac hypertrophy), nervous system (neuron degeneration), male and female reproductive system (testis atrophy, oligosperma, prostate toxicity, mammary gland hyperplasia and uterus atrophy), hepatic system and endocrine system (adrenal gland hypertrophy). Mapping AR together with its chemical interaction partners onto Diseases and Organ and tissue pathologies module identified AR role in prostate neoplasia and prostate carcinoma toxicity that corresponds to AR antagonist therapeutic application. On AR example we showed that our tool can contribute to a better understanding of therapeutic potential of a target inhibition and quick target toxicological assessment.
2098 De-risking Preclinical Drug Development by Transcription Factor Activity Profiling

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Drug toxicity is a leading cause of attrition at late stages of development and a major risk factor for post-marketing withdrawal. Most challenging is the detection of idiosyncratic drug toxicity (IDT). In contrast to dose-dependent intrinsic toxicity, which is usually detected in animal studies, IDT occurs rarely and unpredictably and is often detected only at the post-marketing stage. Assessing IDT risks during preclinical development is extremely difficult due to the lack of a clear mechanistic understanding of IDT pathogenesis. Here, we describe a cell-based approach that predicts IDT liability by assessing perturbations of signaling pathways. Using a multiplex reporter assay (the FACTORIAL™), we assessed the activity of transcription factors (TFs) that connect the cellular signaling pathways with regulated genes. The FACTORIAL™ assay produced TF activity profile (TFAP), a quantitative signature of cell response to a drug. We have evaluated 32 IDT drugs from 12 therapeutic classes with known drug-induced liver injury (DILI) liabilities. We found a common trend: at low concentrations, the TFAPs reflected the primary drug activity, but, beyond certain concentration thresholds (C_{OFF}), these TFAPs transformed into different signatures that indicated “off-target” effects. We estimated that therapeutic concentrations (C_{MAX}) of most drugs in vivo did not reach the off-target thresholds, consistent with the lack of intrinsic toxicity. However, most-DILI-concern drugs were substantially closer to the off-target activity thresholds as compared to their safer counterparts (median C_{MAX}/C_{OFF} of 0.135 and 0.0067, respectively, P<0.00005). That explains an increased IDT risk for most-concern DILI drugs in rare susceptible individuals. Furthermore, we found that drugs from different therapeutic classes shared common off-target TFAP signatures. Querying our database of reference TFAP signatures showed that the most frequent off-target TFAPs matched the signatures for mitochondria inhibitors and inducers of oxidative stress. Therefore, TF activity profiling provided clear quantitative metrics enabling an early-stage assessment of risks and mechanisms of IDT. Supported by the NIH grant R44GM125469.

2099 Characterization of a Modified CNS Tetrad Safety Test in Sprague Dawley Rats

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The cannabinoid (CB) receptor has been considered a potential therapeutic target for multiple indications, however clinical candidates targeting the CB1 receptor showed signs of unwanted CNS activity that resulted in their termination, indicating a need for an early screen that might predict these effects during the lead development phase of drug discovery. The CB tetrad test consists of a number of behavioral assessments (spontaneous activity, catalepsy, body temperature, and thermal sensitivity) designed to assess drugs that induce CB-1 receptor mediated CNS effects. The CB receptor agonist, WIN55,212-2 (WIN), tetrad response was characterized in male Sprague Dawley rats, and the inhibitory effects of the centrally active CB-1 inverse agonist, Rimonabant (RIM), established. Both maximal dose response and the time of peak effect of WIN were established. Adult male Sprague Dawley rats (n=12/group) were administered a single dose of WIN by intraperitoneal (IP) injection at dose levels of 1, 5, 7.5, 10 or 20 mg/kg. Tetrad assessments were evaluated prior to and at 0.5, 1, 2, 4, and 24 hours following dose administration. WIN demonstrated expected increased catalepsy and decreases in spontaneous activity, body temperature and thermal sensitivity at all dose levels. Responses were generally both time and dose dependent with WIN response peaking at 0.5 hours post-dose, with no increase in response or duration of response at dose levels above 10 mg/kg. The inhibitory effect of RIM on the WIN-induced tetrad response was subsequently evaluated. RIM was administered by oral gavage at 0.5, 10, or 30 mg/kg, 2.5 hours prior to the administration of 10 mg/kg WIN IP (n=12/group). Timing of dosing for RIM and WIN targeted the peak exposure of RIM to occur at the time of peak response of WIN (0.5 hours post-dose). Tetrad assessments were performed prior to and at 0.5 hours following WIN dose administration. RIM effectively inhibited the WIN-induced CNS effects in a dose responsive manner with no significant increase in inhibition at dose levels above 10 mg/kg. These data demonstrate the potential utility of the tetrad model in revealing the central-mediated effects of CB1 agonists and antagonists that may be under consideration as drug candidates in multiple therapeutic areas.

2100 A Higher-Throughput Acute Slice Micro-Electrode Array Can Be Used for Neurotoxicity Safety Assessment

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Micro-electrode arrays (MEAs) offer many distinct advantages for measuring neuronal and cardiac electrophysiological activity in vitro. The flexibility and efficacy of MEAs offer powerful solutions for drug discovery, safety pharmacology, and toxicology screening. Here, we demonstrate the power of a high-sensitivity MEA engineered for acute brain slices for improving the efficacy and reproducibility of neurotoxicity screening in acute hippocampal slice preparations from mice. We measured network spike activity and decomposed frequency analysis of field potential oscillations (analogous to EEG) in response to compounds known to elicit seizure-like activity. Acute hippocampal slices from 6-8 week old mice were assessed for epileptiform activity in response to compounds that are likely to elicit synchronized network activity typical of seizure-like activity (convulsants). We demonstrate the capabilities of the highly sensitive MED64-Quad system, a novel medium-throughput MEA engineered for acute or cultured slice applications in assessing neurotoxic risk from acute mouse brain slices. Spontaneous firing and the decomposed frequency components of the local field potentials were measured in response to 4-Aminopiridine, Pentyleneetetrazole, Picrotoxin, Picloram, Acetaminophen and Strychnine. We demonstrate the power of the MED64-Quad system in detecting the small amplitude of the spike though high signal to noise ratio. Several measures of number of spikes, interspike interval (ISI), spike amplitude, co-efficient of ISI and CV of spike amplitude were calculated among the test compounds. Three principal components of the spikes were identified. Slow wave frequency components were observed in response to 4-AP and pilocarpine. These results of this study indicated that the MED64-Quad system, in conjunction with acute hippocampal slices from mouse, is a useful assay for screening epileptiform activity, which is a useful neurotoxic assay for screening safety risk of investigational compounds.

2101 Preclinical Safety Assessment of JM4, a Novel 19-Amino Acid Peptide, for Traumatic Brain Injury and Multiple Sclerosis

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JM4, a low molecular weight 19-amino acid nonhemopoitetic erythropoietin (EPO) peptide, is being studied for possible traumatic brain injury and multiple sclerosis indications in humans. GLP toxicology studies in rat and dog were conducted to support its clinical evaluation. JM4 was administered by intravenous slow bolus (1 to 5 minutes) once daily for 28 consecutive days followed by 14 day recovery period. Vehicle control article or JM4 was administered at doses of 15, 75, or 300 (rat) and 15, 45, or 105 (dog) mg/kg. In rat, JM4 did not cause any significant effect on mortality/morbidity, clinical observations, body weights, food consumption, functional observational battery (FOB), ophthalmic examinations, micronuclei assessment, anti-drug antibody analysis, and clinical and anatomic pathology. Non-test article related nine mortalities in male rats (2 control, 2 low dose, and 5 high dose) were observed during the treatment phase. In dog, nonadverse dose dependent increases were observed in emesis, excess salivation, and fecal status changes (including diarrhea, soft feces, and mucoid feces) that were attributed to JM4 administration. JM4 did not produce any significant changes in body temperature, body weight (or body weight gain), food consumption, ECG waveforms or intervals, respiratory rate, ophthalmic examinations, anti-drug antibody analysis, or in clinical and anatomic pathology. Systemic clearance of JM4 was rapid with mean half-lives between 0.085 to 1.477 hours (rat) and 0.128 to 0.406 hours (dog). Based on the endpoint evaluated, 300 mg/kg/day dose of JM4 (mean C_{max} 634,500 ng/mL and mean AUC 179,500 hr*ng/mL) in rat and 105 mg/kg/day (mean C_{max} 468,500 and mean AUC 65,600) in dog were considered NOAELs. Taken together, these non-clinical studies of JM4 support its further clinical evaluation. Supported by NCI-Leidos Contract No. HHSN26120080001E, and NINDS under BrIDGS Program.
**2102 Drug Permeation Assessment through Reconstructed Vaginal Epithelium: The Case of Econazole**

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The Medical Device Developmental Tool (MDDT) program adopted includes the use of commercially available human reconstructed vaginal models and considers as endpoints tissue viability and histology. US FDA is taking into consideration in vitro permeation studies (IVPT) to assess the bioequivalence of dermatological products adopting the technical and quality criteria of the OECD in vitro permeation absorption method (TG 428). In the study, the permeation of econazole, antifungal compound, has been assessed on the reconstructed Human Vaginal Epithelium (HVE) (Episkin, Lyon, France) to investigate the relevance of the in vitro approach in gaining information on active bioavailability, absorption kinetic and its biological impact on the viable tissue. The transmucosal permeation has been assessed on a formulation including 1% econazole for a 24h exposure. Econazole analytical method by HPLC-ULPC has been validated on the formulation and biological samples before performing the study. The receptor fluids have been collected at 8h and 24h and at 24h the apical residuals, tissue homogenates were performed, collected and analysed. The permeation study was complemented by information on the integrity of the mucosal model after product treatment by performing Lucifer Yellow (LY) assay, trans-epithelial-electrical-resistance (TEER) measurements and histomorphological analysis. Results indicated that compared to the amount of product applied, 4% and 27.4% of the antifungal compound is permeated through the tissue after 8h and 24h exposure, respectively. At 8h the morphology of the tissues treated with econazole and their barrier function resulted not significantly different from tissues treated with saline solution. On the contrary, at 24h data suggested a modified permeability and potential toxicity of the compound on the tissue with a significant decrease of electrical resistance and an increase LY flux. The histomorphological analysis confirmed the tissue damage after 24h exposure. Results were compared to available data and for the 8h timepoint they were consistent with in vivo and in vitro literature data. The in vitro approach proposed appears of interest within MD biological evaluation and bioequivalence studies with the aim to replace the in vivo Rabbit Vaginal Irritation test currently performed for pre-market approval, reinforcing sustainability of the approach with respect to EU Directive 2010/63 on the protection of animals used for scientific purposes and the US FDA MDDT Program.

**2103 Human Reconstructed Mucosal Models to Assess Drug Permeation**

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US FDA has been extending its consideration for in vitro methodologies to permeation studies (IVPT), drug safety and toxicology. Compared to skin permeation study for which skin explants are available from surgical waste, the availability of mucosae (non-keratinized tissues) from humans is a not a sustainable option. To gain relevance and predictivity vs humans, 3D reconstructed human mucosal models are the most promising alternative to be introduced to assess as first instance the penetration kinetics and passage of new drugs and ingredients. In the present study, the permeation of caffeine used as model drug has been assessed on the Reconstructed Human Oral Mucosa (RHO) (Episkin, Lyon, France) to gain information on caffeine penetration, kinetic and impact on the living tissue. The quality and acceptance criteria of the OECD TG 428 (in vitro percutaneous absorption method) have been followed. The transmucosal passage of a 2% caffeine in water solution on RHO model has been performed during a realistic exposure of 2h. After 15, 30, 60 and 120 min the receptor fluid has been collected and caffeine content quantified by HPLC-ULPC. The permeation study was complemented by information on the integrity of the tissue after treatment with the drug by performing Lucifer Yellow (LY) assay, trans-epithelial-electrical-resistance (TEER) measurements and histomorphological analysis to confirm that the integrity of the biological model during the permeation study is maintained. The results indicated that after 2h the 93.3% of caffeine is permeated through the tissue compared to the caffeine initially applied and a permeability coefficient which nicely correlates with literature data was calculated. After 2h of treatment, TEER measurements, LY permeation data and the H&E stained sections showed at 24h no significant differences from the control tissues exposed to saline solution. These results highlight the relevance and robustness of the in vitro approach for the evaluation of the permeability of drugs and substance based medical devices delivered through mucosal caviities. The use of reconstructed mucosal models in combination with a multiple endpoint analysis approach introduce a reproducible and versatile tool for screening purposes and limits as first step the scientific and ethical concerns regarding the use of animals in toxicology.

**2104 Antifertility Effect of Antituberculosis Drugs Combinations Containing Ethambutol or Streptomycin in Male Rats**

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The antifertility effect of antituberculosis drugs (ATD) in combination, which contains ethambutol (EMB) and EMB alone, in male rats has been reported by us earlier. Analysis of potential effects of different ATD combinations (with or without EMB) on the male gonads is urgently required for the development of more effective and safer regimens for treatment of infertility.

The aim of the study was comparative investigation of the two ATD combinations, which contained isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA) and EMB or streptomycin (STM), effects on male rats’ fertility and spermatogenesis parameters, as well as antenatal development of their offspring. Male rats were divided into 3 groups: 1 - control; 2 - 1st combination (EMB, RIF, INH, PZA); 3 - 2nd combination (STM, RIF, INH, PZA). ATD were given at maximal doses used in clinic. The degree of destructive changes in the spermatogenic epithelium following administration of both ATD combinations was almost the same, but time of spermatozoa motility after administration of combinations 1 and 2 decreased as compared to control 77 and 34%, respectively. Osmotic resistance of spermatozoa following combination 1 administration decreased 65%, whereas combination 2 did not have a significant effect. Combination 1 caused increase of lipid peroxidation (LPO) in testes (13%) and epididymis (38%), whereas after use of combination 2 intensification of LPO was recorded only in testes. In male groups with combinations 1 and 2 administration fertility indexes were 9.09 and 78.12, respectively. Thus, the replacement of EMB with STM reduced the toxic effect of ATD on gonads to a certain extent. The results of our study broaden the theoretical basis for understanding of male infertility possible causes.

**2105 Preclinical Development of a Novel Intramuscular Male Contraceptive: Dimethandrolole-17β-Undecanoate (DMAU, CDB-4521)**

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The need for an effective and reversible male contraceptive is still largely unmet. DMAU, a testosterone analogue, exerts both androgenic and progestational effects by releasing its active metabolite DMA, which suppresses testosterone production and spermatogenesis. The goal of this work was to develop DMAU as a long-acting injectable male contraceptive. Four preclinical studies in male nonhuman primates (NHPs) were conducted. First, after a single intramuscular (im) injection of 10 mg/kg DMAU to NHPs (n=4), serum levels of DMAU and DMA were maintained for 231 and 266 days, respectively. Second, weekly im injection of 1 mg/kg DMAU for 3 months to NHPs (n=3) caused no adverse effects. DMAU or DMA did not show significant plasma accumulation with this regimen and no toxicity was observed. Third, im administration of 10-30 mg/kg DMAU chronically suppressed spermatogenesis on fertility is reversible. Fourth, a single im dose of 30 mg/kg DMAU was well-tolerated in NHPs and suppressed fertility as indicated by decreases in testosterone and seminiferous tubular hypospermatogenesis indicating suppression of fertility without any adverse effects. Testosterone reduction in testosterone and seminiferous tubular hypospermatogenesis were divided into 3 groups: 1-control; 2-3\(^{rd}\) combination (STM, RIF, INH, PZA); 4-embo- and HHSN275201500002I; ONPRC P51OD011092. The need for an effective and reversible male contraceptive is still largely unmet. DMAU, a testosterone analogue, exerts both androgenic and progestational effects by releasing its active metabolite DMA, which suppresses testosterone production and spermatogenesis. The goal of this work was to develop DMAU as a long-acting injectable male contraceptive. Four preclinical studies in male nonhuman primates (NHPs) were conducted. First, after a single intramuscular (im) injection of 10 mg/kg DMAU to NHPs (n=4), serum levels of DMAU and DMA were maintained for 231 and 266 days, respectively. Second, weekly im injection of 1 mg/kg DMAU for 3 months to NHPs (n=3) caused no adverse effects. DMAU or DMA did not show significant plasma accumulation with this regimen and no toxicity was observed. Third, im administration of 10-30 mg/kg DMAU chronically suppressed spermatogenesis in a reversible manner without adverse effects, supporting its potential as a long-acting male contraceptive. The first-in-man Phase 1 im study of DMAU is currently ongoing. Supported by NIH-NICHD Contracts HHSN27520090001C and HHSN275201500002I; ONPRC P51OD011092.
2106 The Impact of High Heart Rates on Quantitative Electrocardiography Evaluation: Can Overlapping P and T Waves Be Accurately Assessed?

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Accurate marking of electrocardiogram (ECG) waveforms (P, Q, R, S, and T waves) is integral to meaningful cardiovascular evaluation on nonclinical safety studies. Ketamine, a dissociative anesthetic, is often used to restrain non-human primates (NHP) for multi-lead (ML) ECG collections. Physical or chemical restraint results in higher heart rates, causing ECG waveforms of some NHP to overlap where P waves blend with preceding T waves (P on T); P on T may reduce data quality, cause laboratory rework, and add additional animal stress. This study optimized procedures for ECG evaluation and assessed the impact of including P on T waveforms in ECG evaluation. ECG data (1 min means) were collected from ketamine-restrained NHP using Jacketed External Telemetry (JET) with laboratory procedures that mimicked ML ECG collection. NHP JET data (n=24) were collected to determine if ECGs were optimally evaluated prior to ketamine administration and up to 20 min postdose. PR, QT, and QTc intervals across a range of heart rates were compared between two datasets: one dataset with waveforms from all NHP marked (including NHP with P on T) and another with marks removed from waveforms for the subset of NHP with P on T. The standard time for ML ECG collection (10 min postdose), heart rates were higher by 30 beats per minute (bpm, 20%) compared with predose values and decreased to 21 bpm (14%) at 14 min postdose. By comparison, heart rates in conscious, physically-restrained NHP were 86 bpm (58%) higher compared with heart rates when NHP were unrestrained. Time-course evaluation of heart rate on T indicated that ECGs were optimally evaluated in sedated NHP between 14 to 16 minutes postdose, a time range when no P on T was observed. The impact of marking P on T waveforms, evaluated at 10 minutes postdose, indicated the difference between marking and not marking P on T was up to 2 (3%), 14 (6%), and 13 msec (3%) longer for PR, QT, and QTc intervals, respectively. Taken together, these data demonstrate that ML ECGs collected at 14 to 16 min postdose and when no P on T waveforms collected from ketamine-sedated NHP can be accurately marked, thereby reducing data loss and rework, and increasing the overall ECG data quality on toxicology studies.

2107 High Content In Vitro Cell Monitoring of Adjuvant Chemotherapy Effects in Breast Cancer and Treatment-Related Cardiomyopathy

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Impedance changes of cell-covered electrodes give profound insight into cell proliferation and contractility, even over prolonged time periods, providing significant advantage over standard mostly endpoint cytotoxicity assays. Here, this technology was used for monitoring cell regrowth in breast cancer, after chemotherapy treatment in vitro. As the emerging field of cardio-oncology aims to find a balance between oncologic efficacy and reducing adverse cardiovascular effects, we tested the same treatment on induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs). One of the standard clinical regimens for breast cancer is a combination of cyclophosphamide, Adriamycin (doxorubicin) and 5-fluorouracil (CAF) administered for 4 months. Clinical regimens for breast cancer is a combination of cyclophosphamide, Adriamycin (doxorubicin) and 5-fluorouracil (CAF) administered for 4 months. The high-resolution impedance monitoring provides amenable insights into dynamics of cell proliferation and contraction, for in vitro investigations of adjuvant chemotherapy not only in cancer but in cardiac-oncology field.

2108 Concomitant Effects of Clonidine on QT Interval Duration, hERG Current, Heart Rate and Body Temperature in Cynomolgus Monkeys: QT Correction Formula for Changes in Core Body Temperature

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A method of QT-interval correction for changes in core body temperature (BT) accounting for circadian variations in conscious non-restrained cynomolgus monkeys and based on QT Fredericia formula (QTfC) was recently proposed by our team. To evaluate the performance of this formula and a QT Bazett (QTb) based formula, we investigated the effect of clonidine, an α2-adrenergic receptor agonist, on BT, heart rate (HR) QT, QTb, QTcT, and QTbCt (=(QTb−13.8Bt)/Bt) and QTfCt (=(QTf−13.8BT)/BT) in four telemetry-collared Cynomolgus monkeys. The intrinsic repolarisation effect of clonidine was evaluated on hERG current (I_{in}) in three HEK 293 cells using the whole-cell patch-clamp technique. The greatest changes in BT and QT interval were simultaneously observed at 2 hours after intramuscular injection of clonidine (100 mg/kg). Decreases in BT (36.6 ± 0.6 vs 38.2 ± 0.6°C, p<0.001) and HR (79 ± 10 vs 158 ± 9 bpm, p<0.01) were observed with significant increases in QT (385 ± 217 ± 12 ms, p<0.001), QTb (433 ± 18 vs 350 ± 11 ms, p<0.001), QTfCt (416 ± 24 vs 298 ± 11 ms, p<0.001), QTbCt (406 ± 19 vs 352 ± 12 ms, p<0.001) and QTfCt (382 ± 16 vs 301 ± 10 ms, p<0.001). Clonidine inhibited I_{in} current by 7.8%, 23.8%, 38.8%, 62.5% and 72.9% at 3, 10, 30, 100 and 300 μM, respectively. I_{in} current was established at 30.6 µM. These results highlight a concomitant collection of effects on QT and HR shortening by acting simultaneously on BT, HR, and hERG current. Under our experimental conditions QTbCt performs better than QTfCt in correcting QT for changes in BT. As clonidine produced a slight inhibition in hERG current, significant increases in QTb and QTfCt were still observed after correction for changes in BT. Additional investigations involving other coadministered drugs and measurements of free plasma concentrations are required to fully validate the use of these QT correction formulae for changes in BT.

2109 An Improved Model for Thorough QT (TQT) Clinical Trials That Explicitly Includes RR and Does Not “Correct” QT

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The US FDA requires TQT clinical trials of many new drugs to define their effect on the QT interval with the aim of reducing drug induced arrhythmias. The standard statistical method for TQTs applies a mathematical “correction” to the QT interval based (erroneously) on model equations derived by Bazett (1920) and Fridericia (1925). The standard method subtracts placebo QTc values from the QTc values of the treatment group to obtain ΔQTcT at T_{max}. Nevertheless, the removal of RR from the relationship artificially decreases the variance of QTcT. This potentially leads to incorrect sample sizes and type I or 2 errors. QT correction also artificially increases the values of QT. The standard method assumes that the drug has no important effects on RR; this is frequently not the case. Using publicly available data from an US FDA sponsored trial, I reanalyzed QT and RR values from volunteers given dofetilide, verapamil, ranolazine, and quinidine in a linear mixed effect model. I included RR and uncorrected QT explicitly in the new model allowing for interaction between RR and concentration. The model is: QT ~ Intercept + RR + Concentration + RR:Concentration + 1|Volunteer. To obtain model estimates of the drug effect at 60 bpm, I set RR = 1000 ms in the model solution. I set the value of concentration to the maximum concentration for each volunteer to obtain the maximum difference from placebo. I compared the results with those from the placebo group using Student’s t-test and determined means to obtain the maximum difference from placebo. I compared the results with those from the placebo group using Student’s t-test and determined means to obtain the maximum difference from placebo. 

For in vivo tumor regrowth investigation, H8N8 T3.2 cells were treated with CAF for a second time. Changes in impedance and confluency of these cells, were used as a measure of toxicity with cell viability monitored for 500h, under physiological conditions. Intrinsically dose-dependent effects of CAF clinical treatment: decreased tumor growth and treatment cycle-dependent regrowth of tumor cells was observed. We further investigated putative cardiovascular side effects of CAF mix and paclitaxel and their long-and short-term implications on IPS-ECMs viability. Our results show a dose-dependent negative effect of paclitaxel on the viability of IPS-ECMs, as seen by the base impedance reduction. This was also observed for doxorubicin alone, but not the rest of the CAF compound mix. Paclitaxel and CAF also induced negative changes in cell contraction properties. In summary, long-term high-resolution impedance monitoring provides amenable insights into dynamics of cell proliferation and contraction, for in vitro investigations of adjuvant chemotherapy not only in cancer but in cardiac-oncology field.

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relationship between QT and RR. QT "correction" renders these changes invisi-
ble. Incorrect estimates of the pharmacodynamic effect of a drug on the QT
interval may lead to misinformed regulatory or commercial decisions.

2110 A 4-Week Repeated Dose Toxicity Study of
TPN672 in Beagle Dogs
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TPN672, a novel chemical structure targeting multiple receptors for treating
schizophrenia, is currently in phase 1 clinical trial. TPN672 was effective in
treating phenycyclidine-induced psychosis at doses as low as 0.03mg/kg in
mice. The objective of this study was to evaluate the potential toxicity of
TPN672 in Beagle dogs. TPN672 was orally administered to dogs (5/six/dose)
at doses of 0 (0.5% CMC-Na, vehicle control), 0.1, 0.6 and 3.0 mg/kg once daily
for 4 weeks followed by a 4-week recovery period. Toxicity was assessed by
examining clinical signs, body weight, food consumption, body temperature,
ophthalmoscopy, ECG, blood chemistry, hematology, coagulation, urinaly-
sis, gross pathology, organ weights and histopathology. Key findings were
as follows. All the animals were well tolerated. No obvious changes were
found in hematology, plasma chemistry, coagulation, urinanalysis and prolactin.
Decreased bodyweight was found in both dose males on the first week and
then when that was recovered gradually. Pupillary dilatation, vocalization, tremor
occurred in some animals. Decreased serum glucose was observed at 3 mg/kg.
Slight increase of serum alanine aminotransferase were observed in 3 mg/kg group.
These effects were likely exaggerated pharma-
cological effects of TPN672. Besides, an increase of heart rate was observed for
all dose animals. A slight alopecia of the prostate occurred in males dosed
at 3 mg/kg. These changes were recovered after the 4-week recovery period.
Taken together, we concluded that a dose of 0.1 mg/kg/day was the lowest
observed-adverse-effect level (LOAEL) in both sexes. At the LOAEL, the Cmax
and AUC0-24h of TPN672 at week 4 were 60.5± 35.2 ng/mL and 778.0±642.4
ng·h/mL, respectively, for males and 53.9±9.6 ng/mL and 572.0±149.3 ng·h/
ML, respectively, for females. Compared to the two anti-schizophrenia drugs, no
changes in prolactin levels were found in this study suggesting that TPN672
may have less adverse effects related to hyperprolactinemia in clinical use.

2111 Safety Studies and Preclinical Development of
Novel EPAC Inhibitors as Promising
Therapeutics for Drug-Resistant Rickettsiosis
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Rickettsiae are major human pathogens and potential bioterrorism threats. It
has been forecasted that temperature increases due to global climate change
will lead to more widespread incidence of rickettsiosis, a tick-borne disease.
Although broad-spectrum antibiotics, strains-resistant Rickettsia prowazekii have been developed in some laboratories. Rickettsia australis can be fatal if not treated in a timely manner. It has been demonstrated that cyclic AMP (cAMP)/Epac1 signaling axis plays a key role in con-
trolling various cellular functions in endothelial cells including initial bacterial
adhesion and invasion. The inhibition of cAMP/Epac1 axis in the endothelial
cells is important in the prevention of rickettsiosis. The reported data from
Epac1 knockout mice demonstrated that deletion of the Epac1 gene in mice
protected against a lethal dose of Rickettsiae, making Epac 1 a novel therapeu-
tic target for treatment of rickettsiosis. To this end, our team is developing the first-in-class Epac 1-specific inhibitors as group 1 treatment. About 35 ESIs
were screened for genotoxicity using the in vitro Ames mutagenicity assay
with 3 compounds tested in the in vitro Mouse Lymphoma (MOLY) assay. ESIs
with high in vitro potency and no genotoxicity were tested in in vitro metabo-
ism, as well as in safety and pharmacokinetic studies in mice. Three drug
candidates (ES109, NY0173, and NY0541) with minimal toxicity and desirable
pharmacokinetic profiles were selected to move forward to preclinical safety
assessment in rats and dogs. NY0173 and NY0541 have been identified suit-
able for Investigational New Drug Application (IND)-enabling studies as novel
rickettsioses therapeutics. Here we discuss the safety and preclinical develop-
ment of ESIs leading to the selection of the final two promising therapeutic
candidates. Supported by NIH-NIAID grant 1R01AI111464.

2112 A 9-Month Oral Toxicology, Efficacy,
and Pharmacokinetic Study of
Dimethandrolone-17β-Undecanoate (DMAU, DDB-4521)
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DMAU is being developed as a potential oral male contraceptive. In this study,
toxicity of DMAU and its active metabolite DMA was evaluated. Adult male
Cynomolgus macaques received daily oral doses of 0, 0.3, 1.5, 3 and 0.75/10
mg/kg/day for 9 months followed by a 3-month recovery period. One group of
males received 0.725 mg/kg/day for 6 months followed by 0.75/10 mg/kg/day
during the first 6 months of the study with animals in the recovery groups
gaining moderate to significant weight from baseline. The toxicokinetic (TK)
analysis did not show significant plasma accumulation of DMAU and DMA
during treatment. Peak DMAU plasma levels on Day 1 ranged from 2.68-23.02
ng/mL for doses of 0.3-3.3 mg/kg/day with Tmax ranging from 2-3.4 hr. DMA TK
profiles on Days 190 and 269 suggested steady DMA levels up to 24 hr post
each dose. DMA peak plasma values on those days ranged from 3.25-10.75
ng/mL with Tmax ranging from 2-16.7 hr due to apparent steady DMA plasma
levels. Dose increase to 10 mg/kg/day on Day 190 did not result in elevated
total plasma exposure since there was no appreciable accumulation of DMAU
and DMA over time and inter-dosing periods. The adverse event profile with
DMAU at the oral doses given daily was well-tolerated and the no observed
adverse effect level (NOAEL) exceeded 3 mg/kg/day for approximately 6
months and 10 mg/kg/day for 3 months. The maximum tolerated dose (MTD)
could not be determined since all dose levels were well tolerated. Work sup-
ported by NIH-NICH Contracts HSNS275200900014C and HSNS275201500020.

2113 Investigation of Potential Target-
Independent Uptake Mechanisms of ADC
Induced Thrombocytopenia
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Antibody-drug conjugates (ADCs) are emerging novel anticancer chemother-
apeutics designed to increase the therapeutic index of chemotherapeutics
with more selective delivery of highly cytotoxic agents to cancer cells. How-
ever, non-target mediated hematotoxicity (toxicity to blood cells) mainly
affecting megakaryocytes (platelets) lineage cells, is commonly reported as a
target-independent dose limiting toxicity for multiple ADCs with microtubule
inhibitors in pre-clinical and clinical studies. In this study, we investigated the
lineage-specific hematotoxicity (thrombocytopenia) potential of a non-bind-
ing MMAF based tool ADC using in vitro human bone marrow derived CD34+ hematopoietic stem/progenitor cells. Expansion and differentiation of CD34+
lineage cells into specific hematopoietic lineages (myeloid, erythroid and megakaryo-
cytic lineages) and expression of non-target mediated uptake mechanism
related candidate receptors were evaluated by RT-PCR and flow cytometry
analyses. In general, expression of activating type FcγIRa expression was high in
all three lineages differentiated from CD34+ cells, whereas inhibitory type
FcγIRb expression was observed only in myeloid and erythroid lineages, not in
megakaryocytic lineage cells. Also the rate of non-specific endocytosis (dextran uptake) was relatively low in megakaryocytes when compared to
typical myeloid lineage and other phagocytic cells. Treatment of non-binding ADC
targeting the non-mammalian protein (tetanus toxoid) conjugated to MMAF
with non-clearable mAb linker during different stages of differentiation (7 and
14 days) revealed relatively higher toxicity in megakaryocytic lineage cells. In
addition, pre- and co-treatment with FcγRII blocking antibody significantly
reduced internalization, intracellular free payload release and ADC-induced
megakaryocyte loss. Further testing of different tool ADCs engineered to alter
FcγR binding revealed that mutant IgG (with loss of FcγR binding) contain-
ing ADCs had significantly reduced toxicity in megakaryocytic lineage cells.
Results of this study conclusively confirmed that FcγRII-mediated non-target
mediated uptake in differentiating megakaryocytes is an important mecha-
nism for MMAF based ADC induced thrombocytopenia.
2114 Characterization of a Novel Oxygenating Therapeutic

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Ox66TM is a novel therapeutic with possible uses in oxygenation of blood and tissues. However, the exact structure and mechanism by which the compound may be able to induce oxygenation is not yet understood. This study aims to understand the chemical structure and formula of Ox66TM, thereby opening the way to understanding the mechanisms by which oxygen can be released and absorbed following exposure to the drug particles. In order to elucidate chemical structure and activity, various analytical chemical techniques were used, including energy-dispersive X-ray spectroscopy, infrared spectroscopy, mass spectrometry, and x-ray crystallography. All analytical techniques used a nanosized form of the particle with a particle size of around 600 - 700 nm. Solubility for purposes of mass spectrometry was achieved using a basic solution of ammonia, water and methanol. The results reveal that the compound is amorphous, composed mainly of oxygen and aluminum, and contains a larger and more complex structure than simple aluminum compounds such as alumina, aluminum sulfate, or aluminum nitrate. Furthermore, when dissolved in a basic solution containing ammonia, the aluminum ions exhibit a dynamic chemical behavior where various ligands are exchanged off within a liquid. This study contributes to the understanding of the mechanism of oxygenation of Ox66TM and opens the way to understanding the mechanisms by which oxygen can be released and absorbed following exposure to the drug particles.

2115 Preclinical Evaluation of Pharmacokinetics and Toxicity of AT1965, a Novel Immunotherapeutic Agent

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Triple-negative breast cancer (TNBC) is an aggressive form of cancer occurring in 15-20% of breast cancer patients, with a median survival of 13 months. There is an urgent need for new approaches that are efficacious and safe for TNBC patients. We have designed AT1965, a novel therapeutic with remarkable anti-tumor activity in murine TNBC models as well as potential therapeutic utility in the clinic. AT1965 was predominantly excreted through hepatobiliary and renal routes. The PK of AT1965 determined in 4T1 tumor bearing female Balb/c mice; female Sprague Dawley rats and female Beagle dogs at various doses and schedules reveal that AT1965 exhibited a high exposure, slow systemic clearance and a proportional increase in drug exposure. For safety assessment of biologicals, the non-human primate (NHP) is often selected for longer-term studies if toxicity profiles are ‘similar’ in two species in short term studies. An NC3Rs/Association of the British Pharmaceutical Industry (ABPI)-led international consortium is reviewing the need for two species in regulatory toxicity studies. We collected data on 172 molecules from 18 organisations, including 46 monoclonal antibodies (mAbs), 15 recombinant proteins (RPs), 10 synthetic peptides (SP) and 6 antibody-drug conjugates (ADCs) that followed ICHS6 guidelines. We investigated which molecules used one or two species, and for those using two, whether any reduced to one species during the package. We also collated the incidence of similar target organ toxicities between species in short term (2-13 weeks) First-in-Human (FIH) and long-term studies if toxicity profiles are ‘similar’ in two species in short term studies. An NC3Rs/Association of the British Pharmaceutical Industry (ABPI)-led international consortium is reviewing the need for two species in regulatory toxicity studies. We collected data on 172 molecules from 18 organisations, including 46 monoclonal antibodies (mAbs), 15 recombinant proteins (RPs), 10 synthetic peptides (SP) and 6 antibody-drug conjugates (ADCs) that followed ICHS6 guidelines. We investigated which molecules used one or two species, and for those using two, whether any reduced to one species during the package. We also collated the incidence of similar target organ toxicities between species in short term (2-13 weeks) First-in-Human (FIH) and long-term studies if toxicity profiles are ‘similar’ in two species in short term studies.
2119 Micro-CT and Histopathology Characterization of Atherosclerotic Plaque in Aorta from High Fat Diet-Fed Ovariectomized Apolipoprotein E Knockout Mice

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Atherosclerosis is a systemic, multifactorial disease affecting arteries throughout the body causing severe health consequences and representing a serious concern that can be assessed in nonclinical studies. The objective is to quantify the morphologic changes in atherosclerotic plaques in aortas from high fat diet-fed ovariectomized (OVX) Apolipoprotein E (Apoe) knockout (KO) mice by micro-Computed Tomography (μCT) and characterize associated plaque histopathology. Forty-two Apoe KO and Wild Type (WT) C57BL/6 female mice (Jackson Laboratories) were fed a high fat diet starting at 5-6 weeks of age and were OVX at 8 weeks of age. The procedures involving the care and use of animals were approved by CRL’s Institutional Animal Care and Use Committee. Prior to undergoing a whole-body cardiac perfusion, animals were euthanized at 13, 18 and 26 weeks of age (14/group) by cardiac puncture while under isoflurane anesthesia. The aortas in situ were transferred in formalin, cleaned of adipose tissue and scanned at 10 μm voxel size using a Scanco Medical AG μCT-100. Images were analyzed using 3D morphology to quantify atherosclerotic mineralized and total plaque volume after immersion in a 5% phosphatungstic acid solution. The tissues were then transferred for histopathology examination. OVX Apoe KO mice presented progressive increases in plaque volume and showed statistically significant increases in total plaque volume (+205%, +1346% and +7838%, at 13, 18 and 26 weeks of age, respectively) compared to OVX WT mice. Mineralized plaques were only detected at 26 week of age in OVX Apoe KO mice. Atherosclerotic plaques noted in the aorta were categorized according to Stary-derived types 1 through 5, with a variable number of plaques showing areas of necrosis and cartilaginous metaplasia. Results were consistent with the expected progressive development of atherosclerotic plaques in this model. Micro-CT and histopathological evaluations provided a complementary assessment of atherosclerotic plaque formation and can be used as either as a model for efficacy or as potential safety endpoint for drugs with potential atherosclerotic plaque liability.

2120 Detection of Organometallic Compounds and Their Toxicity in Zebrafish Using ICPMS Metal Equivalents

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Organometallic anticancer drugs are being developed at an increasing rate which drives a need for a high through-put toxicological assessment. The zebrafish model has become a powerful platform for screening large numbers of these novel compounds. In this toxicological screening assay there is a necessity for analytical methods which can detect tissue doses for comparison across eukaryotic systems. Although zebrafish are an attractive assessment tool, because of lower cost, and higher through-put compared with rodents, their low mass (<1g adults and 100 µg eggs) make determining tissue dosages difficult. We hypothesize that measuring metal equivalents using ICPMS after organometallic exposure has the sensitivity to determine zebrafish tissue dose and to establish NOAELS and LOAELS. Two anti-cancer drugs, cisplatin and a novel Ru-based compound, PMC79, were evaluated. Nominal cisplatin concentrations of 0, 3.75, 7.5, 15, 30 and 60µg/mL were evaluated. The corresponding cisplatin tissue doses were 0.05, 8.7, 23.5, 59.9, 192, and 461.9 Pt(ng)/embryo. The cisplatin toxicological endpoint was LC50: 31 mg/L (158 µM) and EC50 for delayed-hatching was 5 mg/L (25.6 µM). The LOAEL was 3.75mg/L (19.2 µM) and the tissue dose was 8.7 Pt(ng)/embryo (44pmoles). The PMC79 water-borne analytically determined were 0.17, 0.44, 0.67, and 0.76 mg/L of Ru, respectively. Viable larvae at the three lowest concentrations had tissue doses of 0.19, 0.41, and 0.68 mg/larvae, respectively. The LC50 was 0.79 mg/L (7.8 µM). The NOAEL was determined to be 0.17 mg/L (1.7 µM) and the LOAEL was determined to be 0.44 mg/L (4.3 µM). Cisplatin and PMC79 resulted in distinctly different lesions, and PMC79 was more potent than cisplatin. The ICPMS method has been successfully applied to 5 additional novel Ru-based anticancer compounds for which the zebrafish LC50 values were established. Coupling a sensitive analytical method with the zebrafish model allows for evaluation of potency and organ system toxicity on a delivered tissue dose basis. This research was funded by NIAES-Rutgers NJ01201, NIH-MIEHS P30 ES050022, and Training Grant T32 ES 007148, FCT (project UID/QUI/00103/2013), FCT/2013 Initiative Project IF/013023/2013 FCT, POPH and FSE - European Social Fund. Ph.D. Grant (SFRH/BD/100515/2014).

2121 Multiplexed Screening of Drug Induced Mitochondrial Dysfunction and Cytotoxicity


Recent years have seen a growing appreciation of the importance of the mitochondria and associated metabolic machinery as sites for off-target drug effects, contributing to safety-related attrition and post-market withdrawals. High-throughput in vitro metabolism assays are of particular importance in both the identification and delineation of such liabilities. However, combining both cytotoxicity and mitochondrial toxicity measurements into a single assay offers an opportunity to both simplify testing and provide more context to the characterisation of drug-induced mitochondrial dysfunction observed after longer term exposures. Here we describe a multiplexed assay measuring both mitochondrial function and cell viability in a single 96 well plate. Mitochondrial function is determined directly using an oxygen consumption assay (MitoXpress Xtra), while cell viability is determined in the same well by interrogating cell membrane integrity using Calcein AM. A simple mix-and-measure workflow prevents cross-talk minimises wash steps, and provides robust metabolism and cytotoxicity read-outs (Z’ >0.67). Method utility is evaluated using a compound panel including Antimycin, Oligomycin, Flutamide, Nefazodone, Tamoxifen and Chloramphenicol. Compound responses are unaffected by multiplexing. Additionally; compounds such as Flutamide, with a known direct mitochondrial liability display a more rapid and pronounced effect on cell metabolism than on viability (IC\textsubscript{50}= 8 μM for oxygen consumption assay, ≥100 μM for viability assay after 48h on HepG2 cells), while generally cytotoxicants such as Tamoxifen reveal similar metabolic and cytotoxic responses. The value of measuring maximal respiratory capacity (FCCP treatment) is also assessed, and in the case of Chloramphenicol, is shown to better enable detection of eroded metabolic capacity. Together these data illustrate that a multiplexed assessment of cytotoxicity and mitochondrial dysfunction is feasible, thereby simplifying safety screening, while differential sensitivity can inform on the specifity of any observe mitochondrial dysfunction.

2122 Use of Cytotoxic TNF Interaction to Classify Drugs According to Their Ability to Cause Idiosyncratic, Drug-Induced Liver Injury: Influence of Classification Modeling and of Categorization Criteria

H. Mollon, R. Roth, and P. Ganey, Michigan State University, East Lansing, MI.

Published results indicate that numerous drugs which cause human idiosyncratic, drug-induced liver injury (IDILI) synergize with tumor necrosis factor-alpha (TNF) in killing hepatocytes in vitro. Using a 24-drug set and logistic modeling of drug concentration-response (cytotoxicity) curves in the presence and absence of TNF in HepG2 cells followed by receiver operating characteristic analysis, we were able to classify drugs according to their IDILI liability with high sensitivity (0.93) and specificity (1.00) (Mairui et al. 2017). In the present study, we compared this approach to a simpler one in which the basis for classification was whether the drug was cytotoxic alone or in the presence of TNF. At concentrations of 10 μM, the resulting specificity and sensitivity were lower than that achieved with the modeling approach. In this exercise and in the Mairui et al. (2017) study, the “true” IDILI+...
2123 Establishing a Toxicologically-Based Level for β-Glucans

C. Schubert, and C. Moudabal,

β-glucans (βGs) are polysaccharide characterized by a variety of different beta-galactosidic bonds, typically 1,3,1,6 glycans. Bonds with branches of either 1,4 or 1,6 linkages that are present in cell walls of a wide variety of prokaryotic and eukaryotic organisms such as yeast, fungi, and seaweed. They appear as potential contaminants in large molecule (LM) pharmaceuticals, primarily from the use of cellulosic filters. In addition, naturally derived raw materials such as sucrose and trehalose during LM pharmaceutical manufacturing. βGs are large molecules and similar to endotoxins (even if weaker) in that they may elicit inflammatory responses leading to potential safety concerns following exposure to pharmaceutical products contaminated with this sugar. While USP has provided limit values for endotoxins in common injectables, this may elicit inflammatory responses leading to potential safety concerns following exposure to pharmaceutical products contaminated with this sugar. While USP has provided limit values for endotoxins in common injectables, there are no such limit values available for βGs. Hence, internal safety limits can be adhered to by developing a toxicologically based level (TBL) for βGs. Published non-clinical data are based on oral and parental testing of various types of βGs including soluble, particulate, and yeast glucan in various animal models. A TBL for βGs was calculated by analyzing toxicologically based levels in cell lines.

2123a A Risk Assessment Pathway (RAP) Map for Setting Impurity Limits for Pharmaceuticals

R. Sandhu, M. A. Maier, R. A. Jolly, D. D. Dolan, E. Lovsin Barle, and J. Berce,

A health-based dose limit set for any substance is a culmination of a myriad of decisions made by risk assessors during the risk assessment process. These decisions address data utilisation, risk assessment technique selection and confidence estimation, among other considerations. Since risk assessments are complex, and decisions made in the face of variable uncertainty, different risk assessors can arrive at different limits even for the same chemical and exposure scenario. In the absence of transparency in evaluation, such differences appear inexplicable and erode confidence in the process and outcomes of risk assessment. To help address this problem, we created a decision support framework for pharmaceutical impurity risk assessment that is reconfigurable in light of existing frameworks that focus on limited mutagenic (e.g., setting a limit in the absence of mutagenicity data). To establish the prototype framework, we first evaluated pharmaceutical impurity guidances from several organizations such as ICH M7, ICH Q3C, Risk MaPP and EMA HBEL guidances as well as peer-reviewed publications focused on developing harmonized methods. We extracted information from these guidances and existing frameworks to generate a map based on key characteristics identified in our analysis: exposure scenario applicability (e.g., route, duration, dose rate), data quality and quantity requirements, substance mode of action properties and regulatory domains. We integrated these elements in a structured format to help decision makers systematically weigh evidence related to objectives, options, and considerations for each key step in the limit derivation process. The framework was evaluated using three case studies of different types of impurities; a potentially mutagenic pharmaceutical intermediate with no data, a buffer present in an intravenous formulation and a leachate. The results showed that the framework was workable, as judged by its ability to assist the user in arriving at reasonable conclusions but required iterative modification with each additional case study. Further refinement of the decision support tool will be achieved by additional testing against case studies with different types of impurities and scenarios. The framework is intended to grow in expressivity and adapt for different compound classes as needed to assist risk assessors to use it to conduct their risk assessments, so that it continually improves with time.

2124 MASOT Education and Outreach Committee Partners with Women in Science and GOALS for Girls to Present Forensic Toxicology Concepts to High School Students

A. Dhaneshwar, D. Hardej, and B. Gonzalez,

On August 10th, 2018 the MASOT Education and Outreach Committee partnered with the Women in Science (WIS) Association from St. John’s University and the GOALS for Girls Program from the Intrepid Sea, Air and Space Museum to conduct a pharmacology and toxicology program for 50 rising 9th and 10th grade girls in the GOALS (Greater Opportunity for Advancement and Leadership in Science) program. The experiment provided for the two 1-hour sessions, called “Forensic Toxicology”, utilized a commercially available kit produced by Innovating Science by Aldon Corporation. The experiment involved the use of simulated urine samples from the substances (alcohol, opiates, cannabis and cocaine) and the use of prescriptive tests such as Simon’s and Marquis Tests for initial detection. Participants worked in groups of 4-5 and were supervised by MASOT Education Committee members and WIS scholars to perform the experiments. The girls were introduced to the concept of forensic toxicology, given information on each of the substances that might be found in the simulated urines and were presented with scenarios before carrying out the experiment. Emphasis was placed on the special care required for forensic specimens to maintain chain of evidence and follow up experiments for definitive analysis after presumptive tests. Participants engaged in all phases of the experiment as they distributed urine to test tubes, added detection chemicals and observed color changes indicative of the substances being detected. The students recorded their results after each experiment. Skills that were learned during these sessions included: proper handling of scientific specimens, accurate application of test agents, observation and estimation of color changes associated with the detection methods and recording and interpreting results. The participants observed positive results with each of the experiments and discussed results obtained from each group. Following the workshop, participants were surveyed to indicate what skills and knowledge were achieved due to the session.

2125 Hands-On Genetics Instruction during a Toxicology High School Summer Program


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 (THEED) program was established to engage high school students interested in pursuing scientific careers. A critical goal of THEED is to provide the basic concepts and skills of designing and conducting toxicology studies including dose-response relationships, histopathology, immunohistochemistry, and genetics. Each year, the program is assessed by the students through pre- and post-surveys. For the past 6 years, the genetics laboratory has received the highest scores in student satisfaction. The genetics exercise exposes students to different methods of modern biological research. They are responsible for (1) extracting and isolating their own DNA (supplied from cheek cells), (2) using polymerase chain reaction (PCR) to amplify their DNA, (3) digesting PCR products, and (4) analyzing PCR products by gel electrophoresis. Phenylthiocarbamide (PTC) is an organic compound that either tastes very strong, mild or is virtually tasteless, depending on the genetic makeup of the taster. The ability to taste PTC is a genetic trait. If a single nucleotide polymorphism (SNP) is present, the receptor still cleaves the DNA and a restriction fragment length polymorphism will separate on an agarose gel. In this case, the student is likely a strong or weak taster of PTC.
the SNP is not present, no cleavage will occur, and the student will likely not be able to taste the PTC. Before unveiling the results, the students taste the PTC paper and compare their taste of PTC to their genetic results. Students observed a strong correlation between genotype and phenotype. Using a Likert scoring system of 1 (low) to 7 (high), students (N=44) rated the genetics laboratory a 5.95 (mean). The principles learned in this activity can be applied to forensic toxicology for use in DNA analysis, medicine to determine genetic predispositions, and animal experimentation such as inbreeding. In summary, a genetics-based laboratory activity exposes high school students to the importance of genotype-phenotype relationships in biology and toxicology. Supported by NEHS T32ES007148, P30ES005022, and U54AR055073.

2126 Engagement of Undergraduate Students in Community-Based Environmental Health Science

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Fieldwork is an integral component of learning for students pursuing science majors, but is often overlooked while performing laboratory research. Fieldwork allows students to actively participate in sample collection, and make direct connections between sample site selection and the outcomes of their laboratory analyses. We provided 12 undergraduate students in the Rutgers Undergraduate Research Program (RURP) with an opportunity to conduct fieldwork as part of their 10-week fellowship, which included interactive sessions on heavy metal toxicity and environmental contaminants. The purpose of this activity was to engage students with a community that has a long industrial history, and concerns regarding the consequences of heavy metal contamination. Of particular concern is exposure to high levels of lead (Pb) in children, which affects the central nervous system and may lead to developmental delays. For this effort, 12 students, 2 teachers, and 1 postdoc fellow worked with 6 members of Isles Inc., a community development and environmental organization based in New Jersey, to sample soil and street dust for Pb contamination in an urban environment. Four sampling sites in Trenton were preselected, although students determined the individual soil and dust sample locations. The sites included a former smelter, a former ink/battery manufacturing facility, and a redeveloped land for housing/commercial use. Students kept field notes on sample characteristics and drew maps of each sample location with proximity to roads, schools, and public parks. ICP-MS analysis revealed widespread elevated Pb levels (>125 ppm) above naturally occurring levels (<10-50 ppm) throughout the study region. Mean Pb levels in soil were 155 ppm (range: 19-566 ppm, n=24) and dust were 125 ppm (range: 14-533 ppm, n=22). The highest Pb levels (>500 ppm) were observed in land around the former Magic Marker/Philco Battery site. Mean Pb levels in soil were 155 ppm (range: 19-566 ppm, n=22) and dust were 125 ppm (range: 14-533 ppm, n=22). The highest Pb levels (>500 ppm) were observed in land around the former Magic Marker/Philco Battery site.

2127 Using Low-Cost Air Pollution Sensors as an Educational Tool in an Undergraduate Science Course

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Studying air pollution – particularly air particles - can be complicated due to the high cost of sampling equipment, the long times required to collect samples, and sensitive balances and environmental conditions needed for weighing. This can be remedied by using air particle monitors, which traditionally are very expensive, costing thousands of dollars per monitor. However, in the past few years there has been a recent influx in the creation of low-cost air particle monitors, which range from $100-$2,500 each, are commercially available, and come in a wide variety of designs and capabilities. It should be noted that currently no low-cost air pollution sensor meet US EPA requirements for regulatory applications, but their use is becoming more widespread to supplement US EPA monitors and for citizen science projects. However, to our knowledge, very little work has been done trying to incorporate these new low-cost air sensors into an educational curriculum, where their cost and ease of use can overcome the previous barriers of studying air particle concentrations in various settings. Therefore, a series of laboratory exercises have been developed for an upper-level undergraduate science course using one type of low-cost particle sensor - the AirBeam. There were 4 main parts to this experiment. In the first part, students compared particulate matter (PM) concentration when they burned scented vs. unscented candles to determine whether adding a scent to a candle influences PM concentration. In the second part, students compared PM concentrations when they burned one vs. three candles to determine whether adding an additional source of PM influenced the overall concentration. In the third part, students used a chemical flame hood to assess how air flow influences PM concentration by burning incense in the middle of the hood and changing the location of the AirBeam to be either “upwind” or “downwind” of the incense. In the last part, the students took the AirBeam to a location of their choosing to assess “real world” concentrations of PM. Previous locations assessed were bathrooms, elevators, near smoking areas, and from e-cigarette vaping smoke. Using this sensor allowed the students an opportunity to measure PM concentrations in real-time using a cost-friendly and user-friendly new tool.

2128 Improvisation as a Teaching Tool to Be Incorporated into Summer Undergraduate Research Programs

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Summer undergraduate research programs are uniquely poised for not only offering authentic “hands-on” laboratory experiences, but also programming to enhance students’ career readiness. Areas of professional development of particular interest are small group activities focused on improving the students’ leadership and communication skills. Improvisation, when used as a small-group collaborative learning exercise, is thought to help students learn how to respond to others, embrace their fears and develop better listening skills. Many of these skills require empathy. With this in mind, we developed a three-hour improvisation workshop involving undergraduate students and their near-peer mentors, all of whom were participants in our summer undergraduate research program. The workshop used a variety of verbal and nonverbal exercises including “Yes and...”, “storytelling”, and “mirror mirror”. To evaluate empathy, the 15 participants completed a self-report questionnaire (known as the Empathy Quotient, EQ) prior to and following the workshop. Total EQ scores and the scores of subscales of empathy; emotional reactivity, cognitive empathy, and social skills were evaluated. Pre-workshop, total EQ scores of the participants ranged from 19-59. However, there was no significant difference between the pre-workshop EQ scores as compared to those obtained post-workshop. At the end of the 10-week program a survey was administered to evaluate all of the program activities. In this survey, 28.6% of the respondents indicated that with respect to enhanced communication and self-confidence, the improvisation workshop helped “a lot” while 57.2% reported that it helped “a little/moderate” while 14.2% reported that it helped “a lot”. Thus, while student participation in a single improvisation workshop did not appear alter the students’ empathy, it may be of value for promoting their self-confidence and communication skills.

2129 ToxMSDT: An Innovative Toxicology Research Education Pipeline Program Targeting Underrepresented Undergraduate Students to the Field of Toxicology

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Exposure of undergraduate students, who have interests in STEM careers, to toxicology as a career choice is uncommon across US colleges and universities. Thus, the depth and breadth of a pool of undergraduates applying to graduate programs in toxicology is limited because students who are otherwise interested may not be aware of toxicology as a career option. This is particularly true for underrepresented undergraduates with toxicology careers and training in toxicology fundamentals and skills that will level their entry into graduate programs. Key objectives of the ToxMSDT program are 1) paired student mentee-mentor teams; 2) Responsible Conduct of Research training; 3) online learning modules (http://www.toxmsdt.com) accessible 24/7 and open to the general public that teach students fundamentals and core competencies of toxicology; 4) face-to-face mentorship and networking by participating in 4 meetings: an Inaugural Workshop at ISU, the annual Society of Toxicology Conference, shadowing at mentor’s work place, and a Capstone Conference at TU where the students present on their experiences in the program. Currently, 5/9 (55.6%) of our senior graduates have joined the pipeline in areas of interest to the NIH; 21/30 (70%) of ToxMSDT graduates are still enrolled as undergraduates.
2130 Undergraduate Education Programs at the Northeast and Ohio Valley Regional Society of Toxicology Chapters: Large Impact for a Low Cost
J. P. Gray1, C. P. Curran2, and L. Williams3. 1US Coast Guard Academy, New London, CT; 2Northern Kentucky University, Highland Heights, KY; and 3Bates College, Lewiston, ME.

The Undergraduate Diversity Program at the national meeting celebrates its 30th anniversary this year. It has impacted a diverse range of undergraduate students from colleges and universities nationwide. However, participation in this program is limited due to significant travel costs, restrictions on conference space, and lack of awareness at institutions without toxicology programs. To increase the pool of undergraduates exposed to toxicology, the SOT Undergraduate Education Subcommittee initiated a pilot project involving two Regional Chapters in 2017 with a total budget of $1,000. The Northeast Regional Chapter of the Society of Toxicology (NESCOT) sponsored the $20 registration cost for 25 undergraduates to attend their annual meeting in 2017. After attending the morning scientific session, the students participated in a breakout session modeled on national SOT programs. Following "Lunch with an Expert", presentations utilizing the "Introduction to Toxicology" slide set by Larissa Williams, liver toxicology by José Manatou, and an active learning opioid exercise by Josh Gray and Larissa Williams were presented. The breakout culminated with a guided tour of the animal facilities at Charles River Laboratories. The OVSOT has a long history of offering undergraduate poster awards and involving undergraduates in mentoring events; however, the geographic region of OVSOT is large. Overall, 44 students attended at a cost of ~$25/student. The following data demonstrate impact for the NESCOT students, most of whom had heard of but not enrolled in toxicology. After teaching the course using different formats, the students were asked to respond to the survey. The survey included questions about their interest in pursuing a career in toxicology and how the course had impacted their understanding of the field. The survey also asked about their impressions of the course format and if they would recommend it to others. The results indicated that the program was effective in helping students learn how to work effectively in teams, which is a key program goal in the Environmental Science program.

2132 Strategies for Effectively Incorporating Team-Based Learning in an Undergraduate Toxicology Course
C. P. Curran. Northern Kentucky University, Highland Heights, KY.

Team-based learning has demonstrated value in increasing communication skills and professional development in numerous fields including medicine, pharmacy and business. As a multi-disciplinary field, toxicology education can also be enhanced by team-based learning (TBL). The challenges to implementing TBL in an undergraduate lecture course can be substantial. High-achieving students often resist sharing responsibilities with fellow students they perceive to be less capable. Time management is another constraint, particularly at institutions with a high percentage of students who need outside jobs to meet their tuition payments. TBL was successfully instituted in an Environmental Toxicology class at Northern Kentucky University through a series of assignments that culminated in a service learning project benefiting a local community partner. Online coursework was used to maximize and track participation by each team member. Anonymous surveys allowed students to provide feedback on individual team members. In-class time was allocated to ensure all team members could meet regularly and receive guidance. Most of the students indicated they would like to have a TlxScholar visit at their institution. These successes indicate that Regional Meetings are appropriate places to hold undergraduate-focused introductions to toxicology and expand the outreach to undergraduates throughout North America. As a result of the pilot project success, the Undergraduate Education Subcommittee expanded the program to fund four regional chapters in 2018.

2133 Establishing Foundational Toxicology Education in Sierra Leone Using Active Learning Modules
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In Sierra Leone, there are concerns regarding the human and environmental implications of prolonged toxicant exposure. Sierra Leoneans are routinely exposed to polluted air and water from everyday practices like cooking, vehicle emissions, and mining. Another major source of exposure stems from unregulated hazardous waste dumps that are burned to create space for agriculture, resulting in uptake of a myriad of toxic compounds into food sources. A primary reason that these conditions persist is due to a lack in educational infrastructure regarding the biological effects of toxicants as well as the exposure hallmark toxicological principles. To address the void in toxicology education, graduate students at the University of Wisconsin-Madison have partnered with Project 1808, Inc., a Madison, WI nonprofit and Sierra Leone community-based organization, to develop a series of modules that aim to introduce college students in Sierra Leone to general toxicology principles. This course also aims to establish awareness to relevant human health and environmental concerns and basic environmental monitoring skills, while encouraging dissemination of this information into the community. The model for developing the toxicology education in Sierra Leone is built around the idea that the modules use case studies and examples that are locally relevant. This pedagogical approach can be effectively implemented in a classroom setting and for students to disperse the information to their communities. This has the potential to impact the health and well-being of the community at large. The modules include lectures accompanied by active learning activities, such as local case studies, field trips, and workshops. This approach allows for the incorporation of local knowledge and expertise, ensuring the relevance and practicality of the content for the students and their communities.

2131 Use of Case Studies to Introduce Undergraduate STEM Students to Environmental Regulations
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Engaging undergraduate students in the learning process. The BSPH in Environmental Health requires students to complete a course titled Environmental Regulations. After teaching the course using different formats, the use of case studies to explain the background and origins of the regulations was found to be successful. The students were given case studies that were relevant to their interest and were asked to respond to the survey. The survey included questions about their interest in pursuing a career in toxicology and how the course had impacted their understanding of the field. The survey also asked about their impressions of the course format and if they would recommend it to others. The results indicated that the program was effective in helping students learn how to work effectively in teams, which is a key program goal in the Environmental Science program.
2134 Publishing Trends of Graduate Students in an Interdepartmental Toxicology Program over a 6-Year Period

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Publishing data in peer-reviewed journals is a fundamental part of graduate training in toxicology. Completion of first author papers enables students to transform their dissertation into publications. Likewise, participation as a co-author on a publication reflects the increasing need for team-based science. The purpose of the present evaluation was to assess publishing trends across MS, PhD, and dual degree PhD students at Rutgers, The State University of New Jersey, who graduated from an interdepartmental toxicology graduate program between 2012 and 2018. Data were collected from pubmed.gov using student and mentor last names and matched with programmatic data regarding degree type, eligibility and appointment to a T32 training grant, and gender. Publication records from a total of 37 students (7 MS, 25 PhD and 5 dual degree) were analyzed. Dual degree students included those individuals who received their clinical degree (MD, PharmD, VMD) at Rutgers or another US institution. The percentage of students with at least one publication from their graduate training included 71% of MS students, 88% of PhD students and 100% of dual degree students. Within these groups, the average number of publications were 0.83 for MS students, 3.6 for PhD students, and 5.6 for dual degree students. Of all 127 publications evaluated, 47% were first author publications. Analysis revealed that those PhD and dual degree students appointed to the T32 training grant (N=18) had an average of 4.8 papers/student compared to an average of 2.6 papers/student for those students who were eligible, but not appointed to the T32 grant (N=8). No differences in average publication number between genders were observed. A review of the subject for the journals selected for publication revealed that 60% of the 127 papers appeared in Pharmacology/Toxicology Journals compared to other journal types (such as Physiology or Molecular Biology).

In conclusion, periodic assessment of publication trends within a graduate program is important for continual improvement and refinement of training goals. Supported by NIH T32ES007148 and P30ES005022.

2135 International ToxScholar: Promoting Toxicology Careers Globally

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The goals of the SOT Education Committee’s International ToxScholar (ITS) Award are to expand awareness of toxicology and promote toxicology careers in developing countries through interaction of toxicologists with undergraduate and graduate students. Since 2010, 27 ITS visits and 45 host institution visits involving scientists from 20 countries have been completed. In 2018, the focus was on promoting careers in toxicology, and 3 scholars visited 11 institutions. Dr. Gurjot Kaur (University of Konstanz, Germany) spent several days visiting 4 institutions in India that are without current toxicology syllabi, presenting to a mixture of undergraduates, masters and doctoral students. She also had an event with high school students focusing on air and water pollution and careers in science and toxicology. Dr. Logeswari Ponnamy (Zoets Inc., USA) travelled to 4 rural institutions in India with the aim of informing veterinary students about toxicology as a specialty. She discussed careers (domestic and abroad) in toxicology, the graduate school application process and the resources and membership opportunities available through SOT. Since completing the visits, 12 students have contacted Dr. Ponnamy for mentoring and more details on applying to USA-based toxicology programs. The final visit was by Dr. Haiyan Tong (US Environmental Protection Agency), who visited institutions in China. She presented on career paths in toxicology at institutions and the Chinese Environment & Health Conference, and connected with over 50 graduate/undergraduate students with an interest in toxicology. Following their visits scholars submitted a final report and shared their experiences in SOT’s Communiqué. These blogs have been very popular, with the first two scholars’ blogs (posted March 2018) garnering 1313 and 999 hits, respectively. These numbers are considerably higher than in previous years, which had under 1000 total hits. The final blog was posted recently (September 2018) and has already received 45 hits. The combination of multiple visits and the success of the blog postings highlights that this program continues to expand awareness of toxicology in developing countries. The Education Committee is committed to increasing the number and diversity of countries visited by scholars applying for this award, which are key to promoting toxicology globally.


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An OECD Guidance Document No. 286 on Good In Vitro Method Practices (GIVIMP) for the development and implementation of in vitro methods for regulatory use in human safety assessment was recently endorsed. This guidance document applies to in vitro methods already accepted by the OECD. In China, this is therefore primordial for the implementation and acceptance of in vitro alternatives methods. Since 2011, L’Oréal initiated the EpiSkin™ Skin Irritation Test (SIT) implementation program in China, 61 scientists received formal trainings and the method has been established in 34 organizations including authority, industry and testing service laboratories. Taking the multi-center study (MCS) of in vitro SIT as example, we demonstrated a standard method establishment process in good alignment with GIVIMP. The quality of EpiSkin™ was assured by Shanghai EPISKIN Biotech providing relevant safety information for transport, use and disposal (GIVIMP 1.2, 5.3). All tested chemicals, commercially available from certified suppliers, were selected from scientific publications that supported the adoption of the test method into TG 439 (GIVIMP 4.2, 6.1, 8.4). The formal step-by-step training according to EpiSkin™ SIT SOP was provided to 4 authority labs including 2 scientists in each lab (GIVIMP 1.1, 2.6, 7.1, 7.2, 8.2), those 4 labs together with L’Oréal conducted the MCS. Six chemicals as training set were performed independently by each scientist strictly following the SOP. By showing qualified results, scientists were allowed to conduct the MCS of 2 blindly coded chemicals described in TG 439. A powerful and simple statistic tool, control trend charts for all tested negative and positive controls were used to monitor batch reproducibility and operators’ performance (GIVIMP 2.3, 2.4), obtained >90% within-lab reproducibility in all 5 labs and 95% inter-lab reproducibility (GIVIMP 8.3). The overall predictive capacity was 70% specificity, 94% sensitivity and 82% accuracy met the criteria defined in TG 439 (GIVIMP 8.3, 9.5). In conclusion, the EpiSkin™ SIT implementation program in China, exemplifies the practical way in which the GIVIMP guidance can assist interested parties in the transfer and establishment of in vitro approaches, and also pave the way towards future scientific recognition and acceptance of in vitro alternative toxicology OECD accepted testing methods in this fast-developing country.

2137 Reducing Exposure to Lead-Based Ammunition to Improve Child Health

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The US Centers for Disease Control and Prevention (CDC) has declared that there is no safe level of lead exposure, particularly for children. Well-recognized exposure routes include water and paint from homes (built prior to 1978), but exposure may also occur through the use of lead-based ammunition for hunting, target practice, or recreational shooting. Exposure to lead occurs when the gun is fired and particulate or lead-based propellant is vaporized, which can be inhaled or contaminate skin and clothing. Lead toxicity is due, in part, to its propensity to substitute for calcium in the body. Because of their rapid bone growth, children will absorb about 50% of the lead ingested, in contrast to only 10% among adults. Lead causes a wide range of adverse effects, but one of the most serious is a reduction in intellectual development, leading to a lifetime of damage. In the US, there are approximately 20 million shooters using an estimated 20,000 shooting ranges and about 1,700 junior military training groups. Most of these groups use lead-based ammunition, which is particularly concerning for individuals under the age of 21; studies have documented cases of high blood lead levels among adolescents that result from shooting range exposures. One way to protect the health of these most vulnerable individuals is to require the use of only unleaded ammunition for users under the age of 21. We will discuss the strategies and feasibility of protecting the health and wellbeing of young gun users by restricting the sale and use of lead-based ammunition for those under 21 years of age. We have an ethical responsibility to protect our children from the health hazards of lead exposure.
Phosphine (PH₃) is a potent, odorless, and colorless toxic gas generated from commercially available metal phosphide pesticide tablets. Due to these factors, PH₃ is recognized as a toxic industrial chemical (TIC). While phosphine's precise mechanism of toxicity is unknown, it may act as a mitochondrial toxicant through blockade of the electron transport chain. Humans exposed to PH₃ present an array of symptoms, with some of the most clinically significant being the delayed development of respiratory distress, cyanosis, and edema. To elucidate the mechanism(s) behind phosphine’s toxic effects, a genomic analysis was performed on adult female Sprague-Dawley rats exposed to either PH₃ (16,500 ppm x min) or filtered air. The heart, lungs, liver, kidney, and whole blood samples were collected from exposed animals at 1, 3, 6, or 24 hours post-exposure. Total RNA was isolated from the tissue/blood samples, processed using 3’ in vitro transcription, and analyzed via microarray. Differentially expressed genes were identified using Partek Genomics Suite and mapped to biological and toxicologically relevant pathways using Ingenuity Pathway Analysis (IPA) software. Microarray analysis revealed organ- and time point-specific alterations in gene expression following PH₃ exposure. PH₃ exposure induced gene expression changes in cardiac tissue associated with cardiac cell death pathways and sinus node dysfunction at 1 hour post-exposure, differentiation of muscle cells at 3 hours post-exposure, and hypertrophy of heart at 6 hours post-exposure. Lung tissue gene expression was associated with primary pulmonary hypertension. To confirm these findings, PH₃ exposure was induced at 1 hour post-PH₃ exposure. Genomic analysis of PH₃-exposed rodents has identified potential molecular mechanisms leading to cardiac injury and death following PH₃ inhalation. Additionally, these data will be used to identify candidate therapeutic targets and mathematical countermeasures for PH₃-induced toxicity.

**Swine Model of High Dose Sulfur Mustard Inhalation, and Improved Outcome after Rescue with Fibrinolytic Treatment**

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Inhalation of high levels of sulfur mustard (SM) has been shown to result in death from severe airway obstruction by luminal casts of fibrin. No antidote or specific treatment exists to prevent mortality after SM exposure. A rodent model of severe SM inhalation has been developed, and a promising fibrinolytic therapeutic successfully tested, showing great efficacy at improving survival and recovery outcomes associated with this form of pulmonary damage. The Animal Rule will require that treatment efficacy be shown in a disease-relevant (large) animal model that mimics the human condition. For this reason, we developed a Yorkshire swine model of severe SM inhalation exposure, and tested the same fibrinolytic therapy for efficacy. Sedated/intubated swine were exposed to 15 mg/kg dose (at 12h) of SM vapor via inhalation using a newly developed exposure system, then awakened/extubated. Clinical signs were monitored continuously. Morbidity was assessed every 15 min by oxygen saturation (Spo₂), heart rate, auscultation, respiratory distress score, and arterial blood gas (ABG; hourly) and complete blood count (CBC) assessment. Fibrinolytic therapy was dosed via sedation/free breathing protocol within 1 hour of exposure (<50 mmHg). After necropsy (12h), lungs were fixed and evaluated for cast obstruction and histopathology. All control animals (n=4) exposed to high dose SM died by 10 hours, and had concurrent severe hypoxemia (Spo₂ <75%; PaO₂ < 40 mmHg), severe work of breathing (retractions, open mouth breathing, and coughing), and abnormal auscultation findings (ronchi, expiratory wheezing, diminished breath sounds) prior to humane euthanasia. At necropsy, all control animal lung lobes had severe airway obstruction by fibrin casts (70-100%), confirmed via histology. CBC showed increased white blood cells (>28x10⁹/ul) and neutrophils (>20x10⁹/ul) by 6h, and mild lymphopenia. All animals receiving fibrinolytic therapy (n=3) survived to study endpoint (12h), and showed clearance of airway casts, and improvement in many other morbidity measures. High dose SM inhalation in swine causes respiratory failure and death due to airway casts. Treatment with fibrinolytic therapy in this model shows great efficacy, with improved survival and other endpoints. Fibrinolytic treatment shows promise in improving outcome after high dose SM inhalation injury.

**Regulation of Macrophage Phenotype by Farnesoid X Receptor during Nitrogen Mustard-Induced Lung Injury**

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Nitrogen mustard (NM) is a cytotoxic vesicant known to cause acute lung injury which progresses to fibrosis. This is accompanied by increases in proinflammatory cytokines, M1 anti-inflammatory/wound repair macrophages in the lung. Therapeutics aimed at mitigating lung injury and inflammation. In these studies, we analyzed mechanisms regulating macrophage phenotypic activation, focusing on the role of pulmonary lipids, which are dysregulated following NM exposure. Farnesoid X receptor (FXR) is a bile acid-activated nuclear receptor involved in lipid homeostasis. It has also been shown to regulate inflammatory responses. In these studies, we showed that expression of FXR, along with two of its targets, ApoA and ApoE, was upregulated in lung macrophages following NM exposure. To analyze the role of FXR in macrophage activation, we used FXR⁻/⁻ mice. WT and FXR⁻/⁻ mice were treated with control (PBS) or NM (0.08 mg/kg, i.t.). Bronchoalveolar lavage (BAL) and lung tissue were collected 14 d later. NM caused histopathologic alterations in the lung including inflammatory cell infiltration, septal damage and epithelial thickening. These were more prominent in FXR⁻/⁻ mice. Additionally, in FXR⁻/⁻ mice, but not WT mice, we observed evidence of fibrosis as assessed by Gomori trichrome staining. This correlated with exacerbated increases in BAL protein and cell content. Immunohistochemistry showed that expression of heme oxygenase-1 (HO-1), a marker of oxidative stress, and ADP-ribosylation factor-like GTPase 11 (ARL11), a marker of M1 macrophage activation, were upregulated in FXR⁻/⁻ mice when compared to WT mice. Conversely, arginase 1, a marker of M2 macrophages and tissue repair, was downregulated. Flow cytometric analysis of lung macrophages revealed the presence of pro- and anti-inflammatory subpopulations following NM exposure; these were reduced in FXR⁻/⁻ mice. These findings demonstrate that FXR modulates the response of macrophages to NM and is involved in controlling inflammation. These findings may be useful in the development of therapeutics aimed at mitigating lung injury and inflammation.
2142 Detection of Galectin-3 and Its Interaction with Microparticles in Airway Surface Liquid Following Exposure of Rats to Nitrogen Mustard

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Exposure to the toxic alkylating agent nitrogen mustard (NM) injures the respiratory tract, which can lead to central airway scarring and fibrosis. The underlying pathogenesis of these fibrotic outcomes is not well defined. Galectin-3 (Gal-3) is a 35-kDa carbohydrate-binding protein expressed on the surface of many cells; recent evidence indicates that Gal-3 may play a pro-fibrotic role in the lung by polarizing macrophages toward a pro-fibrotic M2 phenotype and stimulating myofibroblast activation. In these studies we analyzed Gal-3 in rat lung following NM intoxication. Sprague-Dawley rats were exposed by intratracheal instillation to vehicle (PBS) or NM (0.15 mg/ kg). Rats were euthanized 24 hr later and bronchoalveolar lavage fluid (BAL) collected. SDS-PAGE gel electrophoresis and western blotting demonstrated a significant increase in Gal-3 levels in BAL from NM-exposed rats, when compared to controls. NM was also found to increase levels of cell-derived microparticulates (MPs) in BAL relative to control, as determined by flow cytometry and lactadherin binding. A series of ultracentrifugation and washing steps was performed to isolate MPs from BAL. Western blotting revealed that Gal-3 is associated with MPs. This co-localization was confirmed by incubation of vehicle control BAL (exhibiting negligible Gal-3) with 20 ng of recombinant Gal-3 protein. These findings show that airway MPs are capable of binding soluble Gal-3. Future studies will investigate whether MP interactions with Gal-3 functions as a signaling element for cell-cell communication in response to NM inhalation injury. Supported by NIH AR050732 and ES050202.

2143 Circulating Cell-Free Nucleic Acids in the Pathogenesis of Sulfur Mustard Analog-Induced Acute Respiratory Distress Syndrome

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Circulating cell-free nucleic acids are increasingly being recognized as mediators of injury in a number of diseases. They are known to activate several pathways, including coagulation and inflammation. Exposures of rats to aerosolized 2-chloroethyl ethyl sulfide (CEES), an analog of the war agent sulfur mustard, caused lung injury and increased levels of extracellular nucleic acids in the bronchoalveolar lavage fluid (BALF) and plasma. In this study we have investigated the effect of nucleic acid neutralizing (NAN) agents in mitigating CEES-induced injury. CEES exposure increased respiratory distress and hypoxemia that were significantly improved by NAN agent treatment. Blood gas analysis showed that the NAN agent mitigated CEE-induced decrease in arterial blood pH and PaO2/FiO2 ratio. There were also increases in fibrin and HMGB1 in the BALF indicating lung inflammation and activation of coagulation pathway. These were consistent with a phenotype resembling acute respiratory distress syndrome. Administration of NAN agents also decreased cell-free nucleic acid levels, mitigated lung injury and prevented mortality.

2144 Understanding Tissue-Specific Pathologies following Ocular Exposure to Sulfur Mustard Vapor: Towards Improved Therapeutic Design


Following ocular exposure to moderate or high doses of the chemical warfare agent sulfur mustard (SM), many victims develop a persistent disease known as mustard gas keratopathy (MGK). The pathophysiological origin of MGK is unknown, and diverse therapeutic approaches have completely failed to prevent MGK. It is the primary pathological target of ocular SM injury in the cornea. While the effects of SM on the highly regenerative corneal epithelium are extensively described, the effects on the non-regenerative corneal endothelium are completely unknown. The corneal endothelium is a monolayer of cells that maintain the cornea in a dehydrated state through acting as a semi-permeable barrier and osmotic pump. Disrupted endothelial function results in clinically treatable chronic diseases. Hypothesizing that endothelial toxicity may present a novel injury modality that could explain the idiopathic etiogenesis of MGK, we characterized endothelial integrity and function using a well-described rabbit model of corneal vapor exposure that exhibits acute and long-term sequelae commensurate with human outcomes. Convergent methods demonstrate that ocular SM vapor exposure results in acute endothelial toxicity, gross disruption of endothelial barrier function and long-term endothelial pathologies in MGK eyes. Furthermore, we demonstrate that the extent of the endothelial lesion is correlated with MGK. These data indicate that endothelial toxicity occurs at the right time and with the appropriate pathophysiology to contribute to MGK and furthermore explicate many of the previously confusing aspects of clinical progression in SM-injured eyes. Based on these findings, we have proposed a new model of corneal SM injury. We hypothesize that (a) the endothelial lesion is predictive of corneas that will develop MGK (b) corneal epithelial pathologies in MGK eyes are likely secondary to persistent edema in response to corneal endothelial barrier failure (c) the efficiency of endothelial repair influences whether corneas resolve or develop MGK. This model suggests the potential for new strategies based on inhibition from impaired regenerative capacity, and identifies tissues-specific therapeutic targets. Our current studies are designed to further evaluate this hypothesis.

2145 High-Throughput miRNA Library Screen Reveals HSA-let-7c-5p and HSA-let-7d-5p as Regulators of Sulfur Mustard-Induced Injury in an In Vitro Ocular Model


Sulfur mustard (HD) is a potent vesicant historically utilized as a chemical warfare agent. Acute exposure to HD can produce severe ocular, cutaneous, or respiratory injury. Although this agent has been investigated for over a century, the exact mechanisms of injury are still not completely understood. In this project, a microRNA (miRNA) mimic library was utilized to screen for potential targets that modulate the ocular injury response. A miRNA is a small (approximately 22 nucleotides), non-coding RNA that post-transcriptionally regulates gene expression through binding to partially complementary sequences in the 3' UTR region of mRNAs. High-throughput screening was conducted in an immortalized human corneal epithelial cell line. Cells were transfected with miRNA 48 hrs prior to exposure to a 1.0 x LD50 of HD. Cell viability and pro-inflammatory cytokine production were measured as experimental endpoints 24 hrs after exposure. Of the 1237 targets in the mimic library, 150 reached the significance threshold and were advanced into the mimic/inhibitor pair screen. Targets at this stage were considered hits if both the mimic and inhibitor sequences had significant and opposite effects; twenty-one targets reached this threshold. A dose-response variation of the mimic/inhibitor pair screen was used to validate the remaining targets. Of these, HSA-let-7c-5p and HSA-let-7d-5p had the most robust dose-response effect. Members of the HSA-let-7 family of miRNAs have been reported as regulators of cell proliferation, apoptosis, and the inflammatory response. However, as miRNAs function through partially complementary binding, it is difficult to identify their specific gene targets. Direct administration of the miRNA inhibitor could potentially serve as an efficacious therapy. Further work will be necessary to clarify the pathways regulated by these miRNAs and to evaluate their possible therapeutic benefit in response to HD-induced ocular injury.

2146 Effects of Nitrogen Mustard Gas on Mast Cell Activation


There were a notable number of chemical warfare agents (CWA) used during the Gulf War (1990-1991) and as a result left 25-32% of veterans suffering from Gulf War Illness (GWI). The vesicating agent, sulfur mustard (SM) gas is by far one of the most noteworthy CWA used during this time. SM toxicity symptoms closely relate to those seen in veterans experiencing GWI. Prior research has demonstrated that SM targets the bone marrow and has the potential to influence immune cells including mast cells. Our previous data in mice exposed to SM demonstrated a significant increase in pro-inflammatory cytokines IL-6, IL-1β, TNF-α, and IL-2 followed by infiltration of neutrophils and macrophages in the lung. In the skin, exposure to SM was reported to induce mast cell degranulation. Therefore, the aim of this study was to determine if nitrogen mustard (NM) (a surrogate for SM) exposure promotes activation of mast cells. Studies from our laboratory and others have established nitrogen mustard (NM) as a valuable surrogate to decipher the toxicity effects of SM. For these studies we utilized rat basophilic leukemia (RBL) cells that are commonly used as a representative model of mast cells.

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2149 BIPS, a Type-IV Collagenase Inhibitor, Modulates Epidermal Keratin Expression in Mouse Skin Treated with Sulfur Mustard

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Sulfur mustard (SM) is a potent alkylating vesicant that induces edema, epidermal erosions, inflammation, and prolonged wound repair in skin. Earlier studies have shown that overexpression of matrix metalloproteinase 9 (MMP9) degrades proteins of the extracellular matrix and leads to the separation of epidermis from dermis in skin exposed to SM. We have found that MMP9, a type IV collagenase, disrupts collagen IV (Col IV) a major component of the basement membrane (BM) zone in skin wounds post SM exposure. Col IV in the BM network has been recently reported to be a regulator of epithelial differentiation and keratin expression during wound repair. In the present study, we evaluated BIPS [N-hydroxy-3-phenyl-2-(4-phenylbenzenesulfonyl) propanamide], an MMP inhibitor, on epidermal repair and wound healing following SM exposure in a murine ear vesicant model. Markers examined include histopathologic changes, degree of epidermal hyperplasia, skin thickness, and expression of Col IV, MMP9, keratin 10 (K10, a differentiation marker), and keratin 6 (K6, a wound-inducible marker). We found MMP9 mRNA expression increased 24-168 h post SM exposure. Dual immunofluorescence studies using Col IV α1 and MMP9 antibodies showed BM zone breakdown of Col IV and increases in MMP9 expression in the epidermis and the adjacent dermal matrix of the SM injured skin. Strong keratin 6 expression was evident in the tissue, in contrast, keratin 10 expression decreased. BIPS significantly reduced edema and epithelial thickness by 72 h post-SM exposure. BIPS also significantly downregulated mRNA expression of MMP9. Immunofluorescence studies showed that BIPS significantly reduced MMP9 expression. Col IV expression was similar to the unexposed control skin. Dual immunofluorescent studies also show reduced K6 and increased K10 expression. These results indicate that BIPS, by inhibiting MMP9, effectively restores BM integrity by promoting epithelial differentiation and wound re-epithelialization. Targeting MMP9 may be an effective strategy for countering SM-induced cutaneous injury. Supported by NIH grants ES005022, ES007148 and AR055073.

2150 Nitrogen Mustard Modifies and Cross-Links Wild Type and Mutant p53 in Human Epithelial Cells

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Nitrogen mustard (HN2, mechlorethamine) is a bifunctional alkylating agent commonly used in cancer chemotherapy and as a model vesicant to investigate mechanisms of sulfur mustard-induced tissue injury. Exposure to HN2 causes DNA damage and activation of DNA damage response signaling as a consequence of its ability to modify cellular macromolecules, in particular, DNA. The tumor suppressor protein p53 is a transcription factor known to play a critical role in regulating stress including DNA damage. In the present study, we examined the action of HN2 on p53 expression in several human cell lines varying in p53 status. HN2 caused a concentration- and time-dependent induction of p53 in HaCaT keratinocytes which express mutant p53 (H179Y, R282W), A431 epidermoid cells which also express mutant p53 (R273H), p53 wild type HEK293 kidney epithelial cells and A549 lung epithelial cells. These data indicate that the actions of HN2 in induction of p53 are status independent. HN2 was found to cross-link p53 in HaCaT cells, as well as A431 and HEK cells, forming several high molecular weight complexes. Thus, HN2 can...
modify p53 despite mutations that alter the functional status of p53. In HaCaT cells, the p53 cross-linked proteins rapidly degraded (>50% within 6 h post HN2 treatment) indicating that HN2-induced p53 cross-linking enhanced protein degradation. Mechanisms mediating protein cross-linking were studied using recombinant wild type human p53 cross-linked with HN2. LC-MS/MS analysis revealed that HN2 selectively alkylated Cys135, Cys141, Cys229, Cys238, Cys242 on the protein, forming both monoaducts and peptide loop links. Additionally, HN2 cross-linked Cys124 on one molecule of p53 and Cys229 on a second molecule of p53, forming protein dimers. Taken together, our data demonstrate that p53 is a molecular target for mustard vesicants. Modulation of p53 by vesicants is likely to play a critical role in determining downstream responses to HN2 including DNA repair and this contributes to vesicant-induced cytotoxicity and tissue injury. Support: NIH grants AR055073, NS079294, NS108956, ES004738, and ES005022.

2151 A Comparison of the Dermatotoxicity of Mchelorethamine In Vivo, Using the Mouse Ear Vesicant Model, and In Vitro, Using a Reconstructed Human Skin Model

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Mchelorethamine (HN2) is an alkylating agent which is used in anticaner therapy. Dermal exposure of HN2 is associated with extravasation and tissue blistering reactions (vesicant) which can cause death. A major purpose of the present study was to investigate the time dependent dermatotoxicity of HN2 using the mouse ear vesicant model (MEVM). To this end, our operational definition of dermatotoxicity included tissue responses to HN2 consistent with an increase in the wet weights of mouse ear punch biopsies, an increase in the morphometric thickness of H&E stained ear sections and an elevation in histopathological scoring values for tissue edema, hyperplasia, inflammatory cell infiltration and vesication. The ears of male Swiss Webster mice were exposed to a single dose of HN2 (0.5 μmol/ear) or DMSO and the mice were then euthanized at 15 min or 1, 2, 4, 8, 12 or 24 hr following exposure. Mouse ears exposed to HN2 showed an increase in wet weight, morphometric thickness, edema, inflammatory cell infiltration and vesication at all time points. Tissue vesication sharply increased between 4 and 8 hr after HN2 exposure and remained elevated at 12 and 24 hr after exposure. It is worthy to note that ears treated with DMSO vehicle also exhibited an increase in wet weight and morphometric thickness at 15 min, 1, 2, and 4 hr following treatment; however, these vehicle effects eventually subsided by 8 hr. To determine the extent to which HN2 could cause vesication in a reconstructed human skin full thickness model, a preliminary study was performed using samples of T-skin® treated topically with HN2 (0.5 μmol/ear) or DMSO and biopsied 24 hr after exposure. H&E staining of samples of HN2-treated skin showed signs of tissue injury and decreased viability compared to untreated samples or those treated with DMSO; however, none of the samples showed signs of vesication. The extent to which these types of in vitro models can be used to investigate vesication by nitrogen mustards is therefore worthy of further study. On the other hand, the results obtained here using the MEVM provide a better holistic understanding of the kinetics of vesication, and indicate that time points earlier than 24 hr may be useful in assessing the effects of medical countermeasures to mustards.

2152 Effect of Chemical Warfare Agent Simulants on the Regulation of Endothelial Barrier Function


Endothelial cells (ECs) line the internal portion of the vasculature and are responsible for maintaining homeostasis within the body. This function is accomplished through tightly regulating the passage of both fluid and nutrients from the bloodstream to various tissues. When this regulation is disrupted by inflammation or exposure to specific toxic compounds, fluids and macromolecules will accumulate in the surrounding tissues, resulting in edema. This accumulation of fluids is typically regulated through the paracellular pathway, which is defined as the movement of fluids and macromolecules through gaps formed at intercellular junctions. Several chemical warfare agents (CWAs), including the organophosphates V/G-series nerve agents, have been shown to disrupt the endothelium and induce edema following sub-lethal exposures in experimental animals. Outside of reporting edema during in vivo studies, the direct effects of CWAs on the endothelium is widely unexplored. To do this, we examined the effects of four different CWA simulants on the immortalized dermal microvascular EC line (HMEC-1) through the measurement of cell viability and cellular impedance. The CWA simulants chosen for this study included organophosphates (malathion/maloxon), a vesicant simulant [bis(2-chloroethyl)amine hydrochloride], and a microbial toxin (endotoxin). We then compared these results to those obtained from primary cultured microvascular ECs from skin, lung, and heart to determine if HMEC-1s are suitable for evaluating the vascular toxicity of CWAs. Exposure to increasing concentrations of the selected CWA simulants induced similar patterns of responses in the ECs examined. Our results demonstrate that HMEC-1s are suitable for assessing the vascular toxicity associated with CWAs and allow for the generation of toxicity estimates without the use of primary cultured ECs.

2153 Toxicity and Mechanisms to Identify Therapeutic Targets of Phosgene Oxime Skin Exposure

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Phosgene Oxime (CX), an urticas and nettle agent categorized as a vesicant, is a potential chemical warfare and terrorist weapon. CX exposure can result in devastating toxic effects resulting in high mortality due to its fast penetration and ability to cause immediate severe cutaneous injury. Its easy synthesis makes it a dangerous chemical with both military and terrorist potentials. It could be weaponized with other chemical warfare agents to enhance their deleterious effects and cause prompt incapacitation and death. CX is one of the least studied chemical warfare agents with no effective treatments available. Skin damage associated with CX, necrosis and inflammation following CX-induced exposure to CX and mustard vesicants, e.g. sulfur mustard (SM) and nitrogen mustard (NM), is similar; however, CX also causes severe skin injury with immediate urticaria and blanching as well as mortality. The skin urticaria from CX resembles urticaria caused by allergic and non-allergic reactions to various environmental substances and can occur alone or can be associated with lethal allergic reaction, anaphylaxis. Data from our studies in SKH-1 hairless mice showed that CX exposure, using a more accurate and consistent exposure system, causes mast cell degranulation, and release of histamine, tryptase and TNFα that could activate inflammatory pathways, causes an increase in pro-inflammatory cytokines such as COX-2 and that HMEC-1s are responsive to HN2 including DNA repair and this contributes to vesicant-induced cytotoxicity and tissue injury. Support: NIH grants AR055073, NS079294, NS108956, ES004738, and ES005022.

2154 Amelioration of Nerve Agent Injury

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Recent events in the UK and Syria have shown the importance of medical countermeasures against nerve agents. When considering percutaneous poisoning, it is of interest that nerve agents can cause more severe damage from skin contamination in the skin surface in a timely manner. Nerve agent therapies should be administered upon exhibition of signs and symptoms. Rapid transport to definitive medical care is then the priority. Timely intervention using a suite of medical countermeasures offers the best chance of casualty survival. Dstl is addressing the issue of increasing decontamination efficacy. Methodology has been developed to allow evaluation of decontamination regimens in vitro. The current work compared the UK in service decontamination DKP-1 Mk3 fullers’ earth pad (blot, bang, rub) regimen against a microfiber cloth based regimen after VX nerve agent contamination. Franz type static diffusion cells (diffusional surface area 14.87 cm²) were used. Pig abdominal flank skin was used as a surrogate for human skin. The receptor fluid was 50% aqueous ethanol. 14C-VX (10 μl) was placed onto the centre of the skin surface within the donor chamber. Four minutes post VX application the decontamination regimens were carried out. Receptor fluid samples were taken at regular intervals and 14C was measured by scintillation counting. Measured penetration rates (Jmax, μg cm⁻² h⁻¹) were 25 ± 20 (no decontamination), 0.8 ± 1.0 (DKP-1 Mk3) and 17 ± 21 (microfiber cloth). All values are mean ± standard deviation of n=8 individual animal replicates. Statistical significance (P <0.05, two way ANOVA with Sidak’s multiple comparison test) was shown between
cumulative 14C VX penetration for the no decontamination control and DKP-1 MK3 (from 7 hours) and microfiber cloth decontamination (from 12 hours). The likely discrepancy in decontamination efficacy lies with the skins microstructure. The furrows and wrinkles present in skin make it difficult to decontaminate with the weave of the microfiber cloth. Future work will evaluate a range of decontamination candidates against a range of nerve agents prior to testing in an in vivo model with therapeutic intervention administration. J. Dalton, C.H., Graham, S.J., and Jenner, J. "Effect of exposure area on nerve agent absorption through skin in Vitro" Toxicology in vitro 30 (1) 454-461 (2015) © Crown copyright (2018). Dstl. This material is licensed under the terms of the Open Government Licence except where otherwise stated. To view this licence, visit http://www.nationalarchives.gov.uk/doc/open-government-licence/version/3 or write to the Information Policy Team, The National Archives, Kew, London TW9 4DU, or email: psi@nationalarchives.gsi.gov.uk

2155 Genetic-Based, Differential Susceptibility to Exposure to Combined Organophosphate and Increased Glucocorticoid in a Mouse Model of Gulf War Illness

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In 1990-1991, the USA sent 700,000 troops to the first Gulf War. Approximately 25% of the deployed soldiers developed a chronic multisystem illness with many features of "sickness behavior." This disorder now has been termed Gulf War Illness (GWI) and, remarkably, for those so afflicted, most have symptoms that persist to this day, nearly 30 years later. The cause of GWI has been thought to center on a variety of exposures that occurred in theater, including organophosphate nerve agent (sarin) and insecticides (e.g. chlorpyrifos). We have developed an animal model of GWI that combines exposure to corticosterone as a physiologic stressor mimic with trifluoroleflorphosphate (DFP) (as nerve agent analogue) to mirror some of the exposure/conditions that occurred in theater. The model was developed in the C57BL/6 (B6) mouse strain. The question raised was whether 25-30% of the troops became ill, what about those who did not -- all else being equal? The B6 mouse strain is one of the founders of a large panel of recombinant strains (BxD) derived from crossing the DBA/2 (D2) strain. The mouse model of individual differences in susceptibility to combined OP and high circulating glucocorticoid (corticosterone -- CORT) is to test the D2 strain and several of the BxD strains (and both sexes). The protocol involved adding corticosterone to the drinking water (20mg/kg) of the mice for 7 days followed on the 8th day by injection of diisopropyl-flourophosphate (DFP, 4mg/kg) followed 6h later by euthanasia and harvesting the frontal cortex. The index for neuroinflammation was change in expression of proinflammatory cytokine genes, IL1beta, IL6 and TNFalpha. The results showed that the D2 mice were less sensitive to CORT+DFP than the B6 and that there were large differences among 30 of the BxD strains. We then performed genome-wide mapping of the IL1beta results and found a significant marker (quantitative trait locus) on distal chromosome 7. Searching that area on Chromosome for possible candidate genes, we identified Spondin 1 as a candidate. The gene is cis-regulated and its expression is significantly correlated (r=0.76, p<0.01) with the expression of IL1beta expression under exposure to CORT+DFP. These results show that susceptibility to GWI likely has a genetic component and we will show that further testing of the BXD mice will produce more candidates. We can then identify biochemical pathways that differ and possibly develop treatments and means of prevention.

2156 Neuroinflammation Detected by Longitudinal TSPO Positron Emission Tomography (PET) is Associated with Deficits in Learning and Memory in a Rat Model of Acute Organophosphate (OP) Intoxication [BH1]

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Current medical countermeasures for acute OP poisoning do not protect against chronic cognitive impairment, underscoring the need for preclinical models that enable longitudinal monitoring of novel therapies. Previous studies using a rat model demonstrated neuroinflammation coincident with progressive neuronal necrosis following acute intoxication with the OP diisopropylfluorophosphate (DFP), suggesting neuroinflammatory mechanisms of chronic deficits. The goal of the current study was to determine whether OP-induced neuroinflammation, assessed by PET imaging with the TSPO radioligand [18F]PBR111, was associated with cognitive impairment. Adult male Sprague Dawley rats were treated with pyridostigmine (0.1 mg/kg, im) prior to administration of DFP (4 mg/kg, sc), atropine sulfate (2 mg/kg, ip) and 2PAM (25 mg/kg, im), with a subset receiving a benzodiazepine (BDZ) (5 mg/ kg diazepam, ip or 0.7 mg/kg midazolam, im) 45 min post DFP. TSPO 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 on a Bruker 7T MRI at 7, 28, 65, 91, 182 d post DFP. DFP elicited moderate-to-severe seizure activity in all rats as determined using a modified Racine scale. DFP significantly increased TSPO labeling within multiple brain regions, including the hippocampus, thalamus, amygdala and piriform cortices. Regional quantification of [18F]PBR111 uptake was significantly correlated with baseline TSPO expression and amygdala assayed by c-Fos staining. The results demonstrate that BDZ therapy attenuates, but does not prevent, significant regional neuroinflammation following acute DFP intoxication. These findings suggest this neuroinflammation may contribute to cognitive impairment observed in survivors of acute OP intoxication. Supported by NIH CounterACT program (NS079202).

2157 Crossing the Blood-Brain Barrier to Combat Nerve Agent


Organophosphorus (OP) nerve agents inhibit the acetylcholinesterase (AChE) enzyme, disrupting the hydrolysis of acetylcholine. An excess of acetylcholine can lead to seizure activity, convulsions, respiratory distress, and even death. Oximes reactivate OP-inhibited AChE by detaching the OP from the enzyme. Pralidoxime (2-PAM) is the standard oxime countermeasure used by the US Army; however, 2-PAM is unable to cross the blood-brain barrier, making it ineffective against the central effects of nerve agent. Here, serum carboxylesterase knockout (Es1 KO) mice were used to investigate the in vivo reactivation of OP-inhibited AChE by the novel oximes SwRI-80 and SwRI-144, as compared to 2-PAM. Since carboxylesterase is required for OP hydrolysis, this is a vicious bioscavenger of nerve agent in mice. Es1 KO mice are comparable to a human model of OP toxicity. AChE activity was measured via Ellman’s assay for brainstem, cerebellum, cerebral cortex, hippocampus, midbrain, diaphragm, heart, and skeletal muscle. One-way ANOVAs were performed to control contrasts, which received the nerve agent sarin (GB), cyclosarin (GF), or VX followed by saline, to groups which received the corresponding nerve agent followed by one of three doses (n=8-9 per group) of 2-PAM, SwRI-80 or SwRI-144. 2-PAM significantly reactivated GB-inhibited AChE in heart, and VX-inhibited AChE in heart and diaphragm, but did not significantly reactivate GF-inhibited AChE in any tissues. SwRI-80 had no significant effect against GB-inhibited AChE, but it significantly reactivated VX-inhibited AChE in skeletal muscle and two brain regions (hippocampus and cerebellum) not affected by 2-PAM. Against GF, SwRI-80 also significantly reactivated AChE in skeletal muscle and two brain regions (midbrain and cerebellum) not affected by 2-PAM. SwRI-144 significantly reactivated GF and GB-inhibited AChE in all tissues, except GB-inhibited cerebellum and GH-inhibited GF AChE. Similarly, SwRI-144 significantly reactivated VX-inhibited AChE in all tissues, except cortex. The SwRI compounds appear to cross the blood-brain barrier and reactivates OP-inhibited AChE in various brain regions as well as skeletal muscle not affected by the standard oxime countermeasure 2-PAM.

2158 An Ex Vivo and in Vivo Comparative Study with MMB4 and 2-PAM to Determine Liabilities Associated with Toxic Levels of the Oximes

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Oximes represent the standard of care against nerve agent poisoning, but several studies have shown that they can also induce striated muscle paralysis and cardiovascular changes. MMB4 has been proposed a potentially safer and more efficacious alternative to 2-PAM, the current therapeutic standard. Studies have shown that they can also induce striated muscle paralysis and cardiovascular changes. MMB4 has been proposed a potentially safer and more efficacious alternative to 2-PAM, the current therapeutic standard. OXIMES reactivate OP-inhibited AChE by detaching the OP from the enzyme. Pralidoxime (2-PAM) is the standard oxime countermeasure used by the US Army; however, 2-PAM is unable to cross the blood-brain barrier, making it ineffective against the central effects of nerve agent. Here, serum carboxylesterase knockout (Es1 KO) mice were used to investigate the in vivo reactivation of OP-inhibited AChE by the novel oximes SwRI-80 and SwRI-144, as compared to 2-PAM. Since carboxylesterase is required for OP hydrolysis, this is a vicious bioscavenger of nerve agent in mice. Es1 KO mice are comparable to a human model of OP toxicity. AChE activity was measured via Ellman’s assay for brainstem, cerebellum, cerebral cortex, hippocampus, midbrain, diaphragm, heart, and skeletal muscle. One-way ANOVAs were performed to control contrasts, which received the nerve agent sarin (GB), cyclosarin (GF), or VX followed by saline, to groups which received the corresponding nerve agent followed by one of three doses (n=8-9 per group) of 2-PAM, SwRI-80 or SwRI-144. 2-PAM significantly reactivated GB-inhibited AChE in heart, and VX-inhibited AChE in heart and diaphragm, but did not significantly reactivate GF-inhibited AChE in any tissues. SwRI-80 had no significant effect against GB-inhibited AChE, but it significantly reactivated VX-inhibited AChE in skeletal muscle and two brain regions (hippocampus and cerebellum) not affected by 2-PAM. Against GF, SwRI-80 also significantly reactivated AChE in skeletal muscle and two brain regions (midbrain and cerebellum) not affected by 2-PAM. SwRI-144 significantly reactivated GF and GB-inhibited AChE in all tissues, except GB-inhibited cerebellum and GH-inhibited GF AChE. Similarly, SwRI-144 significantly reactivated VX-inhibited AChE in all tissues, except cortex. The SwRI compounds appear to cross the blood-brain barrier and reactivates OP-inhibited AChE in various brain regions as well as skeletal muscle not affected by the standard oxime countermeasure 2-PAM.
rats. Serial blood samples were taken to determine plasma levels. In isolated rat diaphragms, MM84 exhibited a concentration-dependent reduction of nerve-evoked twitch tension starting at ~2 mM with complete paralysis occurring at 5.4 mM (IC_{50} = 3.2 mM). In vivo, both MM84 and 2-PAM elicited a progressive depression in diaphragmatic and skeletal muscle function, culminating in complete diaphragmatic inhibition. A less steep dose-response was observed for Oxime 15 compared to NIMP and PXN. The plasma exposure values at the start of diaphragmatic decline and complete paralysis were 0.6 and 3.9 mM for MM84 and 0.42 and 1.27 mM for 2-PAM. Therefore, the 'estimated safety index' (a fictional index defined as the plasma concentration ratio at collapse / start of decline) was greater with MM84 (6.6) than 2-PAM (3.0). In contrast, the seizures which can cause damage in the rat brain, our lab investigated substituted phenoxyalkyl pyridinium oxime acetylcholinesterase reactivators (US Patent 9,227,937) that penetrate the rat blood-brain barrier (BBB) in vivo tests with a sarin surrogate (nitrophenyl isopropyl methylphosphonate). When new serine hydrolase targets were found, atropine was added to the study. The results indicated that diaphragmatic depression (most likely due to neuromuscular blockade) is the primary liability of MM84 and 2-PAM in vivo, and 2) indicated that MM84 is safer than 2-PAM. In addition, these experiments demonstrated the feasibility of simultaneously assessing neuromuscular and cardiovascular toxicities in vivo, while showing that the ex vivo phrenic nerve-hemidiaphragm preparations may underestimate in vivo effects.

2159 Organophosphate-Induced Neuropathy in the Rat Hippocampus Is Mitigated by Novel Brain-Penetrating Oxime Acetylcholinesterase Reactivators

M. Dail, C. Leach, R. Pringle, E. Meek, C. Green, and J. Chambers.

The nerve agent sarin and the active metabolite of the insecticide parathion, paraoxon (PXN), inhibit acetylcholinesterase, causing excess synaptic acetylcholine and neuronal damage in the rat brain. Our lab investigated substituted phenoxyalkyl pyridinium oxime acetylcholinesterase reactivators (US Patent 9,227,937) that penetrate the rat blood-brain barrier (BBB) in vivo tests with a sarin surrogate (nitrophenyl isopropyl methylphosphonate). When new serine hydrolase targets were found, atropine was added to the study. The results indicated that diaphragmatic depression (most likely due to neuromuscular blockade) is the primary liability of MM84 and 2-PAM in vivo, and 2) indicated that MM84 is safer than 2-PAM. In addition, these experiments demonstrated the feasibility of simultaneously assessing neuromuscular and cardiovascular toxicities in vivo, while showing that the ex vivo phrenic nerve-hemidiaphragm preparations may underestimate in vivo effects.

2160 Novel Pyridinium Oximes in Combination with 2-PAM Potentiate Survival and Neuroprotection following Organophosphate (OP) Exposure

E. Meek and J. Chambers, Mississippi State University, Mississippi State, MS.

Inhibition of the enzyme, acetylcholine esterase (ACHE), in the central nervous system (CNS) by OPs, including nerve agents and some insecticides, results in overstimulation of the nervous system and may lead to death. Therapeutic response for OP exposures includes atropine, a muscarinic receptor antagonist, and an oxime, 2-PAM in the US, to reactivate inhibited ACHE. Although 2-PAM is an effective ACHE reactivator and can increase survival following OP intoxication, a limitation is its limited ability to cross the blood-brain barrier (BBB) and reactivate inhibited ACHE in the CNS. A series of novel pyridinium oximes have been synthesized to increase lipophilicity and the likelihood of BBB penetration. These novel oximes have shown the ability to cross the BBB, reactivate OP inhibited ACHE, attenuate seizure-like behavior and decrease neuropathology. The most efficacious novel oximes, determined from previous studies, were tested in binary mixtures with 2-PAM to maximize survivability and neuroprotection in rats administered lethal doses of nerve agent surrogates or paraoxon. A sarin surrogate, nitrophenyl isopropyl methylphosphonate, NIMP (0.6 mg/kg), and paraoxon (PXN, 0.8 mg/kg) were administered SC in rats following IM administration atropine (0.65 mg/kg) and binary mixtures of 2-PAM and novel oximes (146 µmoles/kg each) at the onset of seizure-like behavior. Novel oximes in combination with 2-PAM yielded 53-87%, 53-93% and 67-93% for NIMP, NIMP/Oxime 15 and PXN, respectively, while 2-PAM alone yielded 40%, 30%, and 50%. Subsequently, 24-hour survival was determined for mixtures of 2-PAM and novel oximes in guinea pigs (MRLGlobal) challenged with a LD_{50} of sarin. Guinea pigs were monitored and scored for signs of toxicity. Survival for binary mixtures of novel oximes and 2-PAM ranged from 63-87%. Toxic signs scores were lower for animals receiving binary mixtures of 2-PAM and novel oximes, indicating neuroprotection. Preliminary safety studies were conducted in rats on the novel oximes in binary mixtures with 2-PAM (146 µmoles/kg each). Rats were administered oxime mixtures and observed for two weeks. Daily body weights were tracked and serum chemistries were analyzed. No signs of toxicity were observed. Results suggest these oximes have therapeutic potential for OP exposures. Support: NIH U01/NS083430.

2161 Identifying New Serine Hydrolase Targets of a Sarin Analogue and Reactivation with Novel Phenoxyalkyl Pyridinium Oximes

C. Price, M. Dail, and J. Chambers, Mississippi State University, Mississippi State, MS.

Organophosphorus compounds (OPs) are known to inhibit several serine hydrolases, such as carboxylesterases, butyrylcholinesterase, and, most well-known, acetylcholinesterase through which it causes its fatal neurotoxic effects. Reactivating additional OP sensitive serine hydrolases could aid in attenuating these effects. The current in vitro study utilizes activity based protein profiling (ABPP) with fluorophosphoenzyme-biotin (FP) to determine the presence of brain serine hydrolases previously unknown to be sensitive to nerve agent inhibition by the sarin surrogate NIMP (nitrophenyl isopropyl methylphosphonate). When new serine hydrolase targets were found, attempts were made to reactivate them with novel phenoxyalkyl pyridinium oximes (US Patent 9,227,937) being developed in our laboratory as brain-penetrating reactivators. Microsomal fractions of brains from adult male Sprague Dawley rats were incubated with NIMP (in ethanol) to inhibit the serine hydrolases. For reactivation, the oxime in DMSO:ethanol (1:1) was added. The reaction was stopped with SDS loading buffer, heated at 90°C, subjected to 10% SDS-PAGE electrophoresis and probed with avidin-horseradish peroxidase following standard Western blot techniques. Protein bands were visible for non-inhibited serine hydrolases and invisible in inhibited samples. Controls included no FP and heat denatured samples. Of the eight serine hydrolases observed, one occurring at about 60 kDa was reactivated using our novel oximes. This new 60 kDa serine hydrolase target was identified as probably being fatty acid amidase hydrolase (FAAH) due to its interaction with PF-04457845, a covalent inhibitor for FAAH. FAAH is a metabolic serine hydrolase that regulates important signaling molecules of the endocannabinoid system such as anandamide and oleamide. OP-induced inhibition of FAAH and resultant anandamide accumulation has been implicated in being responsible for some OP-induced neurotoxicity. Thus, reactivation of brain FAAH during OP-induced toxicity may contribute to some of the neuroprotection observed for these novel oximes. Support in part from NIH U01/NS083430 and in part from the Center for Environmental Health Sciences, College of Veterinary Medicine, Mississippi State University.

2162 Effect of a Novel Brain-Penetrating Oxime Acetylcholinesterase Reactivator on Sarin Surrogate-Induced Changes on Gene Expression in the Rat Brain

D. Stanford, M. Dail, E. Meek, and J. Chambers, Mississippi State University, Mississippi State, MS.

Novel phenoxyalkyl pyridinium oximes (US Patent 9,227,937) penetrate the blood-brain barrier (BBB) and reactivate acetylcholinesterase (ACHE) in vivo tests with the sarin surrogate nitrophenyl isopropyl methylphosphonate (NIMP). Organophosphates are believed to induce expression of stress genes in the rat brain in addition to ACHE inhibition. If correct, NIMP would upregulate mRNA expression of stress genes as compared to the control group or...
the group exposed to NIMP followed by a novel oxime. A PCR array was used to examine changes in mRNA levels of 84 rat genes known to be regulated by stress and toxic cellular responses. Rats were administered 0.325 mg/kg NIMP or the biocompatible vehicle (Multisol) sc, and one-hour post-NIMP exposure administered novel Oxime 20 or 2-PAM (146 μmoles/kg) in Multisol im. Brains were removed and piform cortex was dissected 2 hours post oxime treatment. Treatment groups were vehicle control (Multisol), NIMP, NIMP/ Oxime 20, and NIMP/2-PAM. Tissues were homogenized, total RNA was extracted, and cDNA synthesized. The cDNA was added to Qiagen’s RT- SYBR Green ROX qPCR Mastermix for analysis using the Qiagen Stress and Toxicity Pathway Finder RT® PCR array on the Stratagene Mx3000P. All test groups were compared to Qiagen’s GeneGlobe Data Analysis Center. NIMP exposure seems to alter gene expression associated with cell cycle and cell death processes. In the presence of Oxime 20, different genes are significantly upregulated relating to oxidative and osmotic stress compared to the NIMP group. Compared to controls, the NIMP/Oxime 20 group had over twice as many significantly expressed genes as did the NIMP/2-PAM group supporting the concept of Oxime 20 efficiently penetrating the BBB whereas 2-PAM fails. Additional evidence that 2-PAM is unable to effectively penetrate the BBB comes from the lack of significant expression changes seen when the NIMP/2- PAM group was compared to the NIMP group. Significant differences were observed, however, when the NIMP/Oxime 20 group was compared to the NIMP group. Supported by Center for Environmental Health Sciences, Mississippi State University, Mississippi State, MS and NIH U01 NS107127.

2163 Inhibition and Reactivation Potential of Novel Phenoxyalkyl Pyridinium Oximes on Rat Serum Butyrylcholinesterase and Acetylcholinesterase
R. Nichols, and J. Chambers, Mississippi State University, Mississippi State, MS.

Oxime reactivators are a critical piece in treating poisoning by organophosphates (OPs), which are nerve agents and some insecticides, that potently inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Prolonged inhibition of AChE leads to seizures, cardiac arrest, and death if left untreated, while BChE can function as a stoichiometric scavenger of OPs. Oxime reactivators are strong nucleophiles and act by removing the OP from the enzyme, thus restoring normal enzyme function. The current oxime platforms lack broad spectrum protection and they display poor brain penetration to protect against OP neurotoxicity. Our laboratories have developed new oxime reactivators that can reactivate BChE to provide a pseudo-catalytic function, and they penetrate the blood brain barrier more effectively to reactivate brain AChE. This study investigated the in vitro reactivation potential of our novel phenoxyalkyl pyridinium oximes (US patent 9,227,937) and 2-PAM on both AChE and BChE after inhibition by surrogates of sarin (phthalimidyldimethylisopropyl methylphosphonate; PIMP) and VX (niproxyphen ethyl methylphosphonate; NEMP) and the insecticidal metabolite paraaxon in rat serum, as well as the inhibitory potential of these oximes over the concentration range of 1-200μM. In a standardized in vitro protocol, high reactivation efficacy was observed toward rat BChE by novel oxime 15 vs 2-PAM for PIMP (78% vs 46%), NEMP (86% vs 8%) and paraaxon (95% vs 32%) while 2-PAM was more efficient at reactivating AChE for all OPs. Relatively high inhibition potency was observed with novel oxime 20 for both BChE and AChE, and low inhibitory potential with novel oxime 15 for BChE and moderate inhibitory potential for AChE. 2-PAM displayed low inhibitory potential for both enzymes. Since oximes can act as anticholinesterases, identifying concentrations that can allow for adequate AChE or BChE reactivation without appreciable AChE inhibition will be of great value. Even though the novel oximes have inhibitory potential for both cholinesterases, in rats at realistic therapeutic levels in vivo these oximes have shown impressive efficacy for survival of OP dosages that produce seizure-like behavior and attenuation of histopathology. Therefore this platform could be a promising alternative approach in treating OP poisoning. Support: NIH U01 NS083430.

2164 Novel Pyridinium Oximes Enhance 24-Hour Survivability against Lethal Organophosphate Dosages in Adult Female Rats
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Anticholinesterase organophosphate (OP) compounds were developed as insecticidal pesticides and are also used as nerve agents in chemical warfare. Treatment against acute OP toxicity includes oximes which reactivate phosphorylated acetylcholinesterase restoring enzymatic activity. The oxime currently approved for use in the U.S., pralidoxime (2-PAM), has limited efficacy penetrating the blood-brain barrier. Our laboratory has developed novel substituted phenoxyalkyl pyridinium oximes (US Patent 9,227,937) designed to more effectively penetrate the central nervous system to enhance survivability and attenuate seizure-like signs. Previous studies with male Sprague-Dawley rats indicated that survivability was enhanced against the nerve agent (sarin) surrogate, 4-nitrophenyl isopropyl methylphosphonate (NIMP) and paraoxon (PON), the active metabolite of the insecticide parathion. Against NIMP, novel oximes 15 and 20 demonstrated odds ratios of 2.3 and 1.8 compared to 2-PAM while novel oximes 15 and 55 increased PXN survivability 5.7 and 2.3 times greater than 2-PAM, respectively. Sex is an important biological variable that must be taken into account when testing efficacy of novel antidotes. In this study, female adult Sprague-Dawley rats were treated with LD₅₀ concentrations of NIMP (0.6 mg/kg SC) or PXN (0.8 mg/kg SC). After development of seizure-like behavior, atropine (0.65 mg/kg IM) and one of four oximes, 2-PAM, novel oxime 15, 20, or 55 (0.146 mmol/ kg IM) or Multisol vehicle was administered. Animals were closely monitored for signs of cholinergic toxicity and 24-hour survivability. Odds ratios and p-values were calculated using 2-PAM as the referent. Survival percentages of animals surviving the 24-hour NIMP challenge dose were 40% for 2-PAM and 60%, 73%, and 33% for novel oximes 15, 20, and 55, respectively. The most effective oxime against NIMP was novel oxime 20 demonstrating an odds ratio of 4.1 over treatment with 2-PAM (p = 0.072). Survival percentages of animals surviving the 24-hour PXN challenge dose were 67% for 2-PAM and 93% for all of the novel oximes. All of the novel oximes demonstrated an improved odds ratio of 7.0 over 2-PAM (p = 0.007). These data indicate that the novel pyridinium oximes enhance survivability against lethal OP toxicity as compared to 2-PAM in adult female rats. Support: NIH U01 NS083430 and U01 NS107127.

2165 Evaluation of Allopregnanolone as Treatment for Nerve Agent-Induced Status Epilepticus in Pediatric and Adult Rats

Nerve agents (NA) are organophosphorous compounds that inhibit acetylcholinesterase, causing a buildup of acetylcholine that can lead to salivation, lacrimation, convulsions, status epilepticus (SE) and even death. Thousands of men, women, and children have been exposed to NAs in the past decade, but the vast majority of NA research in animals has only focused on adult males. In this project, post-natal day (PND) 21, 28, and 70 male and female rats were challenged with sarin (GB) or VX to determine anticonvulsant effective doses (ED50) of the neuroactive steroid allopregnanolone (Allo), which has been reported to stop SE. Rats, previously implanted with electroencephalographic (EEG) electrodes to monitor seizure activity, were administered either GB or VX; five min after SE onset, animals were treated with Allo. ED50 values were determined using an up-down testing method based on four reversals (Dixon & Massey, 1983). ED50 values for Allo-treated PND70 rats ranged from 20-60 μg/kg, with rats exposed to VX generally requiring higher Allo doses to terminate seizures. ED50 values tended to be lower in pediatric animals, ranging from 13.5-31 mg/kg. While Allo is effective in terminating seizures, these ED50s are higher than those of benzodiazepines, such as midazolam (MDZ), which has ED50 values ranging from 1-3 mg/kg in this model. The latency to seizure termination in Allo-treated animals is comparable to that of animals treated with MDZ. This makes Allo a sound choice in treatment of NA-induced SE.

2166 Comparing the Anticonvulsant Efficacy of the Neurosteroids Ganaxalone and Allopregnanolone in a Rat Model of Delayed Treatment of Nerve Agent Intoxication
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Nerve agent attacks on soldiers and civilians by terrorist organizations, militant governments, and assassins have been prominent in recent years. Nerve agents irreversibly block acetylcholinesterase activity, resulting in accumulation of excessive acetylcholine (ACH) at neural synapses. This can lead to a state of prolonged seizure activity, known as status epilepticus (SE). As little as 20 minutes of SE is sufficient to produce frank neuropathology that causes chronic neurological dysfunction. Benzodiazepines, the current anticonvulsant standard of care, become less effective as the time-to-treatment increases, likely due to internalization of synaptic GABA receptors. Neurosteroids (NS), which modulate both synaptic and extrasynaptic GABA receptors, offer a promising mechanism to terminate nerve agent-induced SE at delayed treatment time points. Here we utilize a rat model of soman-
duced SE evaluate the efficacy of the NS ganoxalone and allopregnanolone administered in conjunction with standard medical countermeasures. Adult male rats with cortical EEG electrodes were exposed to soman and administered a NS test treatment or vehicle along with midazolam 20 minutes post-SE onset. SE terminated in 6/9 rats treated with ganoxalone, 4/9 rats treated with allopregnanolone, and 0/10 vehicle-treated rats. The latency to SE termination was 34.0 ± 11.2 min for ganoxalone and 15.8 ± 6.5 min for allopregnanolone. Likelihood of SE termination did not differ between NS treatments (Chi-square, p = 0.34), nor did latency to SE termination (t-test, p = 0.20). However, ganoxalone significantly reduced EEG spike frequency and gamma power relative to controls at multiple time points, while allopregnanolone was only effective at a single time point. Following EEG analysis, fluoroJade B (FJB) staining was used to assess the neuroprotective effects of the two NS. Surprisingly, allopregnanolone reduced the number of FJB+ cells relative to controls in the amygdala, piriform cortex, and parietal cortex, while ganoxalone was only effective in the piriform cortex. In conclusion, both ganoxalone and allopregnanolone offer potential anticonvulsant and neuroprotective benefits, but further optimization of NS structure could enhance both outcomes.

### 2167 Lethality and Acetylcholinesterase Inhibition in the Larval Zebrafish Can Be Used as Screening Endpoints for Assessing Organophosphate Acute Toxicity in Humans


Organophosphate (OP) nerve agents (e.g. Sarin, VX, Tabun) and pesticides elicit toxicity through cholinergic shock via inhibition of the enzyme acetylcholinesterase (AChE). AChE is an enzyme responsible for the breakdown of the neurotransmitter acetyl choline in the synaptic cleft for the regulation of neuronal function. Zebrafish express the highly conserved AChE gene as early as the 5-7 somite stage and progress toward full expression AChE by 7 days post-fertilization (dpf). Five dpf embryos also express Cytochrome P450 detoxification enzymes responsible for biotransformation of many xenobiotic compounds. Zebrafish have gained recent popularity as a potential model for human toxicity in response to environmental chemicals and neuroactive pharmaceuticals. In this study, we sought to develop an acute toxicity larval screening tool for AChE inhibiting compounds. We utilized 6 dpf Danio rerio embryos to examine nerve agent lethality and cholinesterase inhibition relative to well-known OP pesticides parathion (PT) and chlorpyrifos (CPF). In addition, we assessed the OP potent, cyto-dependent desulfonated metabolites of the OPs, paraoxon (PT-O) and chlorpyrifos-oxon (CPF-O). We first compared the 24 hour lethality response to VX with the OP pesticides and the oxon counterparts. VX was ~400x more potent than CPF and over 1000x more potent than PT. We then compared the AChE inhibiting potential of VX relative to the OPs when normalized to the lethal dose. VX induced a 100% inhibition of AChE, which was not observed with the less-active commercial pesticides that caused approximately 75% inhibition (PT) after a 24 hour exposure to the LD50. The oxon derivatives of the OP pesticides were notably more toxic than the parent compound (13x for PT-O and 47x for CPF-O) indicating the importance of metabolism in the 6 dpf embryo model. VX, which does not require bio activation, demonstrated lethality and AChE inhibition potential, more similarly to the active oxons than the parent OPs. Overall, this work shows the potential of the 6 dpf zebrafish to be an acceptable model for nerve agent potency and propensity for AChE inhibition, both of which factor into the human toxicity assessment for emerging threat compounds. Approved for public release; distribution unlimited.

### 2168 Intramuscularly Administered A1 Adenosine (ADO) Receptor Agonist (±)-5'-Chloro-5'-Deoxy-ENBA (cdENBA) Induces Isoelectric Brain State in Rats


Organophosphorus nerve agents (NA), such as soman (GD), irreversibly bind and inhibit acetylcholinesterase, the enzyme responsible for degradation of acetylcholine (ACh). The subsequent overstimulation of cholinergic receptors by this excess ACh can result in sustained seizure activity, or status epilepticus (SE), and subsequent neuropathology. The efficacy of current standards of treatment for NA in controlling seizure activity is dependent on the rapidity of administration. In a mass casualty chemical event, prompt treatment of victims may not be feasible, so new countermeasures capable of terminating SE soon after delayed administration are needed. Past research has shown that activation of A1 ADO receptors can inhibit widespread neuronal excitability, which could aid in seizure termination. cdENBA can induce an isoelectric brain state (indicating strong anticonvulsant efficacy) when administered via intraperitoneal (IP) injection (62 mg/kg) in rats. However, IP injections in the field are not practical, and a more realistic route of administration is needed. Thus, the current study examined the ability of cdENBA to induce an isoelectric brain state when given via intramuscular (IM) injection. Adult male Sprague-Dawley rats with EEG electrode implants were monitored for 30 minutes prior to IM cdENBA injection (45-90 mg/kg) and for an additional 5 hours following injection. Power analysis of EEG data demonstrated that a 50 mg/kg IM injection of cdENBA could induce an isoelectric brain state in a comparable amount of time (2.66 ± 0.98 minutes, N=4) to a 62 mg/kg IP injection of cdENBA (2.60 ± 0.76 minutes, N=6). Quick induction of an isoelectric state could be beneficial in treating GD-induced SE, so these results are promising for further studies of IM-administered cdENBA as an effective NA countermeasure for not only immediate, but also delayed treatment.

### 2169 Delayed Treatment with Midazolam Increases Survival but Is Not Fully Protective against Soman-Induced Epileptogenesis and Neuropathology in Male and Female Carboxylesterase Knockout Mice

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Chemical warfare nerve agents (CWNA) are inhibitors of acetylcholinesterase (AChE) and in animal models lead to status epilepticus and spontaneous recurrent seizures (SRS) and severe neuropathology when treatment is delayed. In addition to binding to AChE, some organophosphorus (OP) CWNA such as soman (GD) also inhibit carboxylesterase (CaE), which acts as a bioscavenger and can greatly affect the severity of the toxicity of OP agent exposure. Unlike humans, rodents have plasma CaE activity. The CaE knockout (ES1/-) mouse specifically lacks plasma CaE and might better model human GD exposure compared to wildtype rodents. Since delayed treatment with midazolam leads to benzodiazepine-refractory SE, we characterized the dose response effects of delayed midazolam treatment in male and female mice, for future use as a model to evaluate combination therapies. In Exp. 1 a sequential stage-wise approach was used to establish a 24 h lethality dose-response curve of GD, in both sexes, accounting for stage of estrus in females. In Exp. 2, mice implanted with telemetry transmitters for electroencephalography (EEG) seizure identification were exposed to 62 µg/kg GD (~4LD50) and treated with an admix (ip) of atropine sulfate (4 mg/kg) and H1-6 (50 mg/kg) 1 min after exposure, and with midazolam (1, 3, or 9 mg/kg/ip) at 40 min after seizure onset. Mice were continuously recorded to evaluate initial seizure duration and SRS and then euthanized 2 weeks after exposure for neuropathology assessment. In estrus were the least susceptible to the lethal effects of GD. Delayed treatment with midazolam increased survival in a dose-dependent manner in both sexes, but was unable to terminate behavioral and EEG seizure activity and did not prevent the development of SRS or neuronal loss following GD exposure. This study demonstrated that delayed treatment of SE with midazolam is not fully protective against the GD-induced epileptogenesis and neuropathology, exemplifying the need for adjunct treatment to midazolam to prevent or reduce effects of GD-induced SE. Research was supported by the CounterACT Program, NIH OD, and the NINDS (Grant 1R21NS103820-01 to LA Lumley-Lange).

### 2170 Characterization of a Mouse Model of Tetramethylenedisulfotetramine (TETS)-Induced Status Epileptics


TETS is a potent convulsant rodenticide that is considered a credible chemical threat agent. In humans, acute intoxication with TETS at high doses can trigger seizures that can progress to status epilepticus (SE) and death. Survivors are at increased risk for developing neurobehavioral deficits. Persistent neuroinflammation has been associated with decrements in cognitive, psycho-motor and affective behavior; therefore, in this study we assessed the effects of acute TETS intoxication on neuroinflammatory responses and behavior in adult male C57BL/6j mice. Mice were injected with nirole (5 mg/kg, ip) 10 min prior to injection with TETS (0.3 mg/kg, ip). Using this exposure paradigm, animals exhibited >1 h of persistent seizure activity as assessed by electroencephalography and behavioral observation. In the absence of rescue therapy, ~90% of the animals died by 24 h post-TETS exposure. Diazepam (1.8 mg/kg, ip) or midazolam (1.8 mg/kg, im) administered 40 min after the initiation of seizure activity increased survival at 24 h post-TETS to > 50%. Correlative...
histology revealed time-dependent reactive astrogliosis and microglial activation as determined by GFAP and Iba-1 immunoreactivity, respectively. The magnitude of the neuroinflammatory response was positively correlated with the duration of seizure. C57BL/6/J mice who survived acute intoxication demonstrated increased anxiety-like behavior and hyperactivity at one week post-exposure, and these phenotypes persisted at one month post-exposure. Collectively, these studies identify the C57BL/6/J mouse as a model that exhibits more robust neurologic responses to TETs-induced SE than the previously characterized NIH Swiss mouse model of TETS-induced SE. These findings suggest that the C57BL/6/J mouse may be a better model for assessing candidate therapeutics for efficacy in protecting against delayed and persistent neurologic sequelae. Supported by the NINDS CounterACT Program (grant US4 NS079202).

2171 Acute Intoxication of Juvenile Rats with Diisopropylfluorophosphate (DFP) Causes Sex-Specific Seizure Behavior and Neuropathology

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Organophosphates (OPs) are used globally as pesticides and chemical threat agents, and cause hundreds of thousands of deaths per year as a result of intentional and accidental poisonings. Acute OP intoxication inhibits acetylcholinesterase (AChE), which can trigger seizures that progress to status epilepticus (SE). Neurotoxicity and death. Efforts to develop improved medical countermeasures against OPs have largely focused on adult male models. However, OP-induced neurotoxicity is known to differ between juveniles and adults, as well as between males and females. Therefore, the goal of this project was to develop a juvenile model of acute OP intoxication that elicits seizure behavior and neuropathology. Juvenile (postnatal day 28) Sprague-Dawley male and female rats were acutely intoxicated with the OP chemical threat agent DFP (3.4 mg/kg, s.c.) or an equal volume of vehicle (saline, s.c.) followed by a combined injection of atropine sulfate (0.1 mg/kg, i.m.) and 2-PAM (25 mg/kg, i.m.). Exposed animals were monitored for seizure behavior during the first 4 h post-exposure. At 1 d post-exposure, all animals were evaluated for cortical AChE activity as determined by the Ellman assay. Body weight, neuroinflammation as determined by quantitative immunohistochemistry of Iba-1 (microglia), CD68 (phagocytic microglia), and GFAP (astrocytes), and neurodegeneration as measured by FJC staining. Both males and females showed significant AChE inhibition. Following DFP intoxication; however, females showed minimal seizure behavior compared to males, who showed pronounced seizure behavior for up to 4 h. Following DFP intoxication, females showed increased body weight whereas males showed decreased body weight relative to sex-matched vehicle controls. DFP males displayed severe neuroinflammation and neurodegeneration in the cortex and hippocampus, but DFP females did not. These findings suggest that sex differences exist in DFP-induced neurotoxicity in juvenile animals, and that seizure behavior likely contributes to neuropathology following acute intoxication of the juvenile brain. Supported by the NIH CounterACT program (NS079202) and a predoctoral fellowship to EAG (NIH GM5676520, Initiative for Maximizing Student Development).

2172 Delayed Adenosine A1 Receptor Agonist (±)-5’-Chloro-5’-Deoxy-ENBA (cdENBA) Treatment Terminates Soman-Induced Status Epilepticus

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Nerve agent (NA) intoxication initiates a cascade of deleterious physiological responses that ultimately, induce a state of unremitting seizure known as status epilepticus (SE). As intoxication progresses, different neurotransmitter systems emerge as targets for terminating SE. Current medical countermeasures act upon specific neurotransmitter systems and, therefore, may not be effective in terminating certain phases of intoxication. Previous research has demonstrated that administration of the adenosine A1 receptor agonist cdENBA (62 mg/kg, IP) one minute after NA exposure prevented seizure onset and negated accompanying neuropathology. In this study, we assessed cdENBA’s ability to terminate SE using a delayed-intervention model that simulates realistic medical response times. Adult Sprague Dawley rats were exposed to soman (1.2 x LD50) and remained untreated (N = 8) or received cdENBA treatment (62 mg/kg, IP) 30 (N = 8) or 60 (N = 10) minutes after onset of SE. Five hours after exposure, 50% of the animals treated at 30 minutes and 100% of the animals treated at 60 minutes were alive, compared to 37% of untreated animals. Frequency analysis of the 5-hour post-exposure EEG of surviving animals showed that SE terminated in 3 of 4 rats in the 30-minute group, and 9 of 10 rats in the 60-minute treatment group. No seizure termination was observed in the untreated group. cdENBA demonstrated a novel ability to terminate SE across a range of immediate and delayed administration time points, making it a strong candidate as a countermeasure for soman-induced SE. Future experiments will identify a window for treatment where the brain can be protected against neurotoxicity and the eNOS pathway. cdENBA will be pursued. The views expressed in this abstract are those of the author(s) and do not reflect the official policy of the US Department of Army, US Department of Defense, or the US government. The experimental protocol was approved by the Animal Care and Use Committee of the US Army Medical Research Institute of Chemical Defense, and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. This research was supported by an Interagency Agreement (IAA) between NIH/NIADDK (IAA Number AOD12058-001-00000) and the USAMRICD (Number A120-B.P2012-02).

2173 A Targeted Approach for Terminating Tetramethylenedisulfotetramine (TETS)-Induced Status Epilepticus and Attenuating Neurotoxicity in Zebrafish


Zebrafish have been used as a seizure model for nearly two decades; however, important questions regarding the mechanisms of seizure termination as well as persistent neurologic effects are not well characterized. Our lab is poised to address these concerns using a targeted pharmacological approach encompassing pharmacologic and advanced genetic techniques, including CRISPR/Cas9. In this study, NGR wild-type zebrafish (Danio rerio) were used to screen small molecule enhancers of GABAergic neurotransmission, including CD111, a putative γ-aminobutyric acid receptor type A (GABA_A, R) subunits for efficacy in terminating seizures induced by tetramethylenedisulfotetramine (TETS), a potent GABA_A, R antagonist and credible chemical threat agent. Larval zebrafish were allowed to develop under normal rearing conditions until 4 days post fertilization (dpf) whereupon individual larva were separated into separate wells of a 96-well plate. Following a 24 h acclimation period, each GABA_A, R subunit-specific PAM and the positive control, midazolam (3 μM), were tested in two treatment paradigms: pretreatment for 20 min prior to the addition of 4 μM TETS, and post-treatment 20 min after 4 μM TETS. GABA_A, R α1 subunit-selective compounds were the most effective in reducing seizure behavior following TETS exposure. LS38417 (1 and 10 μM), NS11394 (1 μM), and TCS1105 (1 and 10 μM) also significantly reduced seizure behavior in larval zebrafish when added following TETS exposure. In addition, α2-selective compounds TCS1105 (1 and 10 μM) and LS73847 (10 μM) reduced seizure behavior near midazolam levels when added post-TETS. Our future objective is to characterize the efficacy of GABA_A, R PAMs for efficacy toward attenuating neurotoxic endpoints following TETS exposure. Through this research, we hope to improve current methods for identifying therapeutics for terminating chemical-induced seizures and protecting against imminent seizure toxicity. Supported by the NIH CounterACT program (NS079202).

2174 RNA Seq Transcriptome Analyses Reveals Genes and Pathways Involved in Acute Exposure to Hydrogen Sulfide

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Hydrogen sulfide (H2S) is a gaseous molecule produced endogenously and in the environment. Acute exposure to high concentration of H2S causes severe brain damage and induces long-term neurological sequelae. The exact mechanisms underlying the H2S-induced dysfunction of central nervous system have not yet been clearly elucidated. RNA Seq transcriptome analysis was performed to identify key elements and pathways that contribute to H2S-induced neurotoxicity. C57BL/6J black mice were exposed by whole body inhalation to 700 ppm for 4, 1, or 0.5 h. At 6 h post TETs, the mice were sacrificed. The rats were fixed on subsequent days to evaluate immediate, early and late responses, respectively. 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 RNA Seq analysis. Acute exposure to H2S induced 283, 193 and 278 DEG (q-value < 0.05, fold-change > 1.5) for immediate, early, and late responses, respectively. Dysregulated biological pathways were further analyzed using Ingenuity Pathway Analysis. H2S...
2175 Antidotal Protection Enhancement of the Cyanide Antidote DMTS by Formulations with Combinadine Derivatives

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Dimethyltrisulfide (DMTS) is an established sulfur donor type cyanide (CN) antidote candidate. The focus of this study is to demonstrate how appropriate formulations and combinations with other CN antidotes having different mechanisms of action, can enhance the antidotal efficacy of the neat DMTS. The following formulation compositions and DMTS doses were investigated: 1) Micellar DMTS (2 mg/mL in PEG2000-DSPE, 12.5 mg/kg); 2) DMTS in emulsion (peanut oil, polysorbate 80, glycerol, water, 20 mg/mL, 100 mg/kg); 3) Poly80-formulated DMTS (15% aqueous polysorbate 80, 50 mg/mL, 25 and 50 mg/kg). The combination partners for the Poly80-formulated DMTS (doses: 25 and 50 mg/kg) were a) Nitrocoibinamide (4NCbi): (dose: 20 mg/kg); b) Aqo-hydroxycobinamide (AHcbi): (doses: 50, 100, and 250 mg/kg). The antidotal efficacy was determined by using the Dixon up-and-down method on mice, and expressed as antidotal potency ratio (APR). The “Dunnnett’s Multiple Comparison Test” was employed for significance determination. The APR values of 1.2 (dose: 25 mg/kg) and 1.5 (dose: 50 mg/kg) of the neat DMTS were significantly enhanced by the investigated formulations. When Poly80-DMTS (doses: 25 and 50 mg/kg) was combined with 4NCbi (dose: 20 mg/kg), significant increases in the APR values were noted. AHCbi enhanced the APR of Poly80-DMTS significantly only at the dose of 250 mg/kg. These studies prove the importance of formulations and combinations for DMTS as CN antidote. The Institutional Animal Care and Use Committee (IACUC) permission number is 15-09-14-1015-3-01.

2176 Partitioning and Elimination Kinetics of the Cyanide Antidote Candidate Dimethyl Trisulfide in Sheep Blood

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Cyanide inhibits cytochrome C oxidase, the terminal oxidase of the mitochondrial electron transport chain, thereby suppressing cellular oxygen utilization and aerobic ATP production. Dimethyl trisulfide (DMTS) is recently well investigated as a cyanide antidote candidate. It is a small, highly lipophilic, naturally occurring molecule, present in garlic and onion in the highest concentrations. Studies have shown that DMTS has significantly higher in vitro sulfur donor reactivity and in vivo antidotal efficacy than the present cyanide therapy thiocyanate. This study focuses on the DMTS partitioning between red blood cells (RBC) and plasma as well as the DMTS elimination kinetics in these blood components. This in vitro blood study clearly demonstrates that DMTS accumulates 6.2 times more in RBC than in plasma. The DMTS elimination is substantially faster in plasma (t1/2 = 1.5 min) than in RBC (t1/2 = 2.2 min). Based on the blood partitioning and elimination kinetics, RBC can be considered as an efficient carrier for distributing DMTS to other organs.

2177 In Vitro and Ex Vivo Models in Medical Chemical Defense Research

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The recent use of organophosphorus nerve agents (OPNA) in military conflicts and for assassinations highlights the threat by these compounds and the need for optimized medical countermeasures (MedCM). As animal experiments are expensive, time-consuming and increasingly under public scrutiny, well-conceived in vitro and ex vivo models are important to pre-select candidate MedCMs. The main target of OPNA is inhibition of acetylcholinesterase (AChE). The use of a dynamic flow-through model allows exposure of erythrocytes, muscle and brain AChE to OPNA and MedCM simulating toxicco- and pharmacokinetics combined with real time determination of AChE activity. In the model, inhibition of AChE was with a combination of HI-6 and obidoxime showed no additive effect but the reactivation spectrum was broadened, which could bridge efficiency gaps until a single reactivator with broader spectrum is available. To address the potential of OPNA- and MedCM-induced dysrhythmias, a multi electrode array (MEA) using hiPS-derived cardiomyocytes and a Langendorff apparatus were established. The OPNA cyclosporin resulted in a dose-dependent increase in corrected field potential duration (600 nsec; 9.4±0.5%), which was fully reversible by atropine but not by HI-6. The approved oximes obidoxime and pralidoxime and the candidate reactivators HI-6 and MMB-4 did not induce dysrhythmia in the MEA and the Langendorff heart. Cholinergic crisis results in smooth muscle contraction. Consequently, a small bowel and lung tissue model were established. For the small bowel model, material from patients undergoing routine surgery was used. Specimens were exposed to the OPNA sarin and treated with scopolamine as the most potent compound with an EC50 of 50 nM. The reactivator HI-6 was more potent than obidoxime (EC50 3.8 µM vs. 197.8 µM). As lung tissue model, precision cut lung slices allow to assess the efficacy of asthma and COPD therapies in OPNA-poisoning after acetylcholine-induced airway contraction. β2-agonists had a negligible effect whereas glycopyrrolate (EC50 15.8 nM) and ipratropium (EC50 2.3 nM) efficiently reversed acetylcholine-induced airway contractions in VX poisoning. Significant effects were also achieved by combining formoterol or salbutamol with atropine, vocating a RCT in patients poisoned by OP pesticides. Complex in vitro and ex vivo models are successfully established and can serve as important tools to accelerate development of MedCM and allow optimized planning of focused in vivo experiments.
Exposure to carbon nanotubes and multi-walled carbon nanotubes (MWCNT) is due to their use as drug carriers and medical devices. These MWCNTs that enter the blood circulation end up in the liver and are trapped in the reticuloendothelial cells. We have reported an increase in high mobility group box-1 (HMGB1) expression in hepatocyte cell line (HC-04) due to MWCNT exposure. HMGB1 is considered a mechanistic marker of liver injury since its release is associated with cellular necrosis and immune cell activation. Ethanol and Acetaminophen (APAP) are commonly used agents that produce liver toxicity during overdosing. The toxic effects of MWCNT during co-exposure to known hepatotoxictants (APAP or Ethanol) is not known. Therefore, the goal of the study is to evaluate the cytotoxic nature of MWCNT during co-exposure with hepatotoxictants in HC-04 cells. HC-04 cells were exposed to MWCNT for a period of 72 hours and subsequently treated with either 15 mM APAP or 360 mM Ethanol for 24 hours. At the end of the study, the cytotoxicity was quantified by measuring the lactate dehydrogenase (LDH) leakage into the media. HMGB1 protein expression was measured by western blotting. Percent LDH leakage due to APAP increased from 24% to 46% compared to the control and to 47% due to co-exposure with MWCNT. Similarly, Ethanol also increased the LDH leakage from 27% to 37% and to 42% during co-exposure with MWCNT. No significant change in LDH leakage was evidenced in groups treated with MWCNT alone. Intracellular HMGB1 protein expression decreased by 0.85-fold due to APAP alone and by 0.65-fold during co-exposure with MWCNT. The decrease in intracellular HMGB1 was consistent with the increase in secreted HMGB1. Secreted HMGB1 increased by 40-fold in APAP group alone and by 73-fold during co-exposure with MWCNT. By contrast, Ethanol treatment alone increased HMGB1 expression by 1.4-fold, but during co-exposure with MWCNT, the increase was only 1.1-fold. No secreted HMGB1 was detected due to western blotting. Taken together, these findings demonstrate for the first time the potential for enhanced hepatocellular necrosis during co-exposure of MWCNT with common hepatotoxictants. The mechanism(s) and functional consequences of HMGB1 release is unknown and is currently being investigated.

### 2186 Assessing the Effects of Dietary Additives, Stress, and Bacteria on Intestinal Function in an In Vitro Gut Model

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Inflammatory bowel disease (IBD), irritable bowel syndrome (IBS) and Crohn’s disease are increasingly common gastrointestinal disorders marked by intestinal barrier dysfunction. Their incidence has increased at a rate similar to the increased food additive consumption. Intestinal disorders have also been linked to stress and gut microbial dysbiosis. A Caco-2 and HT29-EM6 cell co-culture model was exposed to chemically additive-glucose salt. TWEEN 20 (emulsifier), titanium dioxide nanoparticles (TiO2 NP), gluten, and food-grade TiO2 NP from M&M candies and gluten from whole wheat bread following an in vitro digestion. The effects of these additives on intestinal permeability and intestinal alkaline phosphatase (IAP), a gut mucosal defence factor involved in maintenance of intestinal homeostasis, activity were quantified. Inflammation was modelled using TNFα, a pro-inflammatory mediator. A Lucifer yellow permeability assay showed a permeability increase across the model with high glucose (p<0.001), TNFα + high glucose (p<0.0001) and TNFα + food grade TiO2 (p<0.05) as compared to their respective controls. Exposure to food grade form of TiO2, and gluten resulted in a higher permeability than their chemical form. Increasing the model in 1% salt resulted to a 1.5-fold increase in permeability (p<0.01) and higher IAP activity than 0.1% salt. To study the influence of microbiota and diet together, Lactobacillus rhamnosus GG and Escherichia coli were used in combination with the food additives. Bacteria led to a decrease in permeability with high glucose, 1% salt (p<0.0001), 1% TWEEN 20 (p<0.01), the chemical grade TiO2 and gluten (p<0.01) and increased permeability with food grade TiO2 (p<0.01) as compared to their respective controls. Post exposure, bacterial viability was quantified to assess the effect of food additives on bacterial growth. Due to the role of indole in increasing paracellular resistance in epithelial cells, indole production by E. coli was measured using the luminol enhancement assay. Both bacteria showed similar increase in permeability, their mechanisms of action were different. L. rhamnosus resulted in a significant increase in IAP activity with high glucose, indicating a possible correlation between intestinal permeability and IAP. E. coli + 1% salt produced more indole per colony forming unit (mU/cFU) compared to 0.01% salt, suggesting indole production as a possible mechanism by E. coli. While stress and food additives disrupt the intestinal barrier function, intestinal bacteria work towards reinstating the integrity of the barrier in response to certain food additives.

### 2187 Physiochemical Properties of NiO and Ni(OH)2 Nanoparticles Correlate with Cytotoxicity in A549 Cells

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We characterized two forms of nickel nanoparticles (NP), NiO and Ni(OH)2, and delineated cytotoxicity in the A549 mammalian cell line. We measured the relative number of available binding sites on the NP surfaces using X-ray photoelectron spectroscopy (XPS). After both NPs were subjected to 48 hr constant pH dependence, composition reactions at both pH 4.5 and 7.4, samples were vacuum filtered and dried to obtain the powders. The NP powders were then subjected to an XPS system analysis. The XPS integrated peak areas of the O 1s binding core level were used to quantify the relative number of available binding sites for biomolecules to interact with the NP’s surface. Quantification was achieved by noting the physisorbed versus chemisorbed oxygen on the NP surfaces. At pH 7.4, there was a 1.6-fold increase in binding sites available on Ni(OH)2, NPs as compared to those on NIO NPs. At pH 4.5, there was a 5.2-fold increase available on Ni(OH)2 NPs as compared to NIO NPs. The effect of pH and suppression of cell proliferation are inter-related. Cellular injuries correspond with the measured properties of the NPs, with Ni(OH)2 being more toxic than NIO.
The blood brain barrier (BBB) represents a significant hurdle in delivering drugs for treating neurological diseases. To date, amongst many other approaches attempted, polyethyleneimine (PEI) has been the most popular non viral vector for oligonucleotide and gene delivery. However, it has the disadvantages of being cytotoxic to cells, tendency to agglomerate and easily taken up by reticuloendothelial system without appropriate shielding. Hydroxethyl Starch (HES), a biodegradable polymer, has been recently introduced as a shielding agent in conjugation with PEI to deliver genes in vitro and shows promise to be used with PEI for targeted delivery to the brain. However, optimization studies are necessary to elucidate the best combination of HES and PEI in terms of molecular weight, particle size, cytotoxicity and DNA condensation properties. A library of different HES-PEI conjugates having different combinations of molecular weights were synthesized as described previously with certain modifications. The HES moiety in the conjugates were then biotinylated with Biotin-PEG4-Hydrazide, purified and lyophilized. The degree of biotinylation of the conjugates were then assessed by the HABA assay. The optimum conjugates for further study were then selected based on cytotoxicity assay, particle size analysis, zeta potential measurements and oligonucleotide condensation studies with Ethidium Bromide with NFkB decoy ODNs. The studies were done in different N/P ratios (3:1, 6:1 and 9:1) in absence or presence of serum and at different HES-PEI mixtures (50:50 and 10:90). The conjugates shielded the PEI at varying degrees when compared to naked PEI, as seen with particle size analysis and zeta potential analysis. HES-PEI conjugates had significantly lower cytotoxicity compared to PEI alone as seen up to 24 hours. HES520-PEI20 and HES510-PEI20 yielded particle sizes ranging from 200-400 nm, whereas HES510-PEI4 and HES520-PEI4 conjugates were found to have much more favorable particle sizes. However, the HES510-PEI4 particle sizes were larger when mixed with different ratios of PEI compared to HES520-PEI4. HES20kDa-PEI4kDa to be the most optimal conjugate with minimal toxicity suitable for further studies in conjugation with targeting antibody.

Engineered nanoparticles (NP) are used in industry and consumer products; however, we are just beginning to mechanistically understand how inhalation exposure to NP impacts human health. Research efforts have focused on pulmonary endpoints such as fibrosis, allergic-type reactions, and cancer, while the area of viral susceptibility remains less well explored. Work by our group has determined that exposure of human small airway epithelial cells (SAECs) to single-walled carbon nanotubes (SWNTs) increases host susceptibility to influenza A virus (IAV) infection by increasing viral titers and repressing anti-viral gene expression (interferon-stimulated genes (ISGs)). To elucidate mechanisms that contribute to these outcomes, we investigated how SWNTs modulate lipid that influence immune signaling pathways, focusing on sphingosine-1-phosphate, its precursors/products, i.e. ceramide (CER), phosphoethanolamine (PE), and metabolic enzymes, sphingosine kinase 1 (SK1) and sphingosine-1-phosphate lyase (SPL). We hypothesized that SWNTs inhibit expression of SPL, resulting in decreased expression of ISGs, and thus increased viral titers. For our approach, we exposed SAECs to 20 µg/mL SWNTs for 24 hrs, followed by exposure to IAV for 24 hrs. We quantified mRNA expression of SPL, SK1, and ISGs via quantitative real-time PCR and protein expression via Western blotting. Changes in lipids were analyzed by liquid chromatography-mass spectrometry. Gene expression analysis revealed that ISGs (Lifts, IFTM6) are suppressed in SWNT + IAV exposures, while expression of SK1 and SPL are unchanged by the presence of SWNTs. IAV induces transcription of SK1, but not SPL post-infection in both IAV only and SWNT + IAV groups, suggesting that changes in protein level or enzyme activity are important. Levels of PE and CER species were significantly different between SWNT-exposed and control samples. These observations demonstrate a role for SWNTs in perturbing lipid metabolism, with downstream impacts on innate immune pathways. Future work will focus on impacts of inhibition or over-expression of SK1 or SPL on the effects of SWNTs and IAV in SAECs. These studies highlight the important role that lipid metabolism plays in the immune response to viruses and targets these pathways in SWNT toxicity.
Multi-walled carbon nanotubes (MWCNTs) are emerging nanomaterials that are widely used in industrial, engineering, biological and medical researches due to their unique physicochemical, optical and electrical properties. The release of these nanomaterials into the environment poses a potential for human exposure following inhalation and ingestion. The purpose of this study was to assess the in vitro toxicity of four MWCNTs with outside diameter of <8 nm, 13-18 nm, 20-30 nm, and >50 nm and two functionalized MWCNTs (-OH, -COOH) with outside diameter 20-30 nm in a rat intestinal cell model (IEC-6 cells). Pluronic (0.1%), a non-ionic surfactant was used to stabilize MWCNT dispersion. Hydrodynamic diameter and zeta potential were calculated to determine the sonication time at which the MWCNTs were optimally dispersed. Based on the results, the MWCNTs suspended in Dulbecco’s media and 0.1% pluronic were probe sonicated for 15 min before dosing. IEC-6 cells were plated in 96-well plate with 60K cells/well for 24 h before dosing. Media with 10% fetal bovine serum was the negative control and Triton X-100 (0.3%) was the positive control. Cells were then exposed to the MWCNTs at various concentrations (0.3-300 μg/ml) for 24 h. Following incubation, the cells were washed with media and cytotoxicity was assessed using a colorimetric method that measures mitochondrial activity. Only the <8 nm MWCNT displayed cytotoxic effects, inducing 50% cell death at a concentration of 300 μg/ml. All other MWCNTs tested were negative. The results suggest that the outside diameter of MWCNTs is an important factor in the cytotoxicity of these nanomaterials in rat IEC-6 cells. This abstract does not necessarily represent US EPA policy.

**2188** Differential Mitochondrial Perturbations among Primary, Cancerous, and Asthmatic Lung Cell-Types after Exposure to Engineered Nanomaterials

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The use of metallic nanoparticles as additives in consumer and industrial products is rapidly increasing. Specifically, zinc and aluminum are highly utilized as fuel additives and other automotive applications, thus increasing the risk of occupational, consumer, and environmental exposure to these engineered nanomaterials. Zinc (Zn) and aluminum (Al) have been shown to accumulate within mitochondria and perturb mitochondrial processes, however the specific mechanisms of toxic action remain unknown. There is a need to develop toxicological testing paradigms focused on the elucidation of Zn, ZnO, Al, and Al2O3 toxicities. The purpose of this study was to determine the effect of zinc and aluminum nanoparticles, as well as their oxide counterparts, on human mitochondrial health along the electron transport chain (ETC). To determine changes in mitochondrial health, three different epithelial cell-types from the upper airway with varying phenotypes were selected as a test system. The three phenotypes include primary cells (PTBE), cancer cells (AS49), and asthma cells (DHBE). These cell-types were selected to represent a healthy human population as well as two unique sensitive subpopulations. Our hypothesis is that the differential baseline mitochondrial health profiles will be key determinants of induced nanotoxicity. Specifically, the primary and asthma cells are more sensitive to the metal nanoparticle exposures when compared to the cancer cell-type because cancer cells as less susceptible to ETC perturbations through oxidative stress. The mitochondria in each cell-type was characterized before and after exposure to normalize mitochondrial health metrics. Characterization included, mitochondrial morphology, mitochondrial dehydrogenase activity, and gene/protein expression. Differential dose-response patterns in mitochondrial activity were seen in mitochondrial morphology assessments, degradation of cristae structure, decreased mitochondrial dehydrogenase activity, and increased antioxidant response. While all cell-types exhibited changes in mitochondrial health, the primary cells showed the most pronounced alteration of mitochondrial structures, while the asthma cell-type experienced increased proinflammatory responses. The changes in the mitochondrial structure and function in the cancer derived cell-type were minor compared to those observed in the other cells. These results give newfound insight that will influence safety testing guidelines by providing evidence to use more realistic healthy (PTBE) or compromised (DHBE) populations.

**2189** Biomimetic In Vitro/In Vivo Models for Assessment of Hazardous Pulmonary Effects of Nanoparticles

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Potential human exposure to respirable nanoparticles (NPs) has become a major concern with increasing evidence showing that NP pulmonary exposure results in particle deposition in deep lung tissues and causes pathological changes. Such adverse health effects have not been well assessed partially because it is impossible to assess the toxicities of countless NPs using animal models, and limited relevance of in vitro models to evaluate specific toxicities of NPs. To address the challenges, we have developed multiple in vitro models to assess the biological and toxicological activities of well-characterized NPs with the identification of target lung cells of pulmonary exposed NPs from animal studies. Based on established in vivo doses that induce significant pulmonary disorders, physiologically relevant in vitro doses (i.e., 0.02 - 0.2 μg/cm²) of NPs, including carbon nanotubes (CNTs) and iron oxide NPs (Fe₃O₄), were used to evaluate their toxic effects on human lung cells under long-term exposure condition (up to 6.5 months). Present study data showed that NPs were able to induce dose- and time-dependent cytotoxicity, inflammation, fibrogenesis and neoplastic transformation of human lung cells, consistent with in vivo data. Our in vitro studies determined specific particle type- and cell type-dependent NP-induced cell proliferation, anchorage-independent growth, apoptosis evasion, and increased cell migration and invasion. Furthermore, by developing a fibroblast stem cells (FSC)-enriched fibroblast focus model to mimic in vivo fibrogenic response, we demonstrated a dose-dependent increase in fibroblast focus formation and collagen production by primary lung fibroblasts treated with multi-walled carbon nanotubes (MWCNTs). This result unveils a novel mechanism of nanotube-induced fibrogenesis through ALDH-dependent FSC activation. The described in vitro-vivo combination approach will support the utility of in vitro models as rapid screening and predictive tools for risk assessment of nanomaterials.
2191 Biointeractions of Aerospace Relevant Nanomaterials with Human Gut Microbiota in a Human Gut Simulator

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Broad inclusion and incorporation of engineered nanomaterials (ENMs) in consumer goods demands an understanding of the impact such products have on humans. Specific concern has risen regarding oral exposures to dietary ENMs and the subsequent impact on gastrointestinal health via microbial dysbiosis. Employing an in vitro Human Gut Simulator (HGS) system, we have examined interactions of dietary nano titanium dioxide (TiO2) with human gut microbiota. Following HGS seeding and community stabilization, we administered TiO2 over seven days, followed by a seven day recovery period. We measured changes to cell density, community structure, metabolic end products, and predicted community functional capacity. Addition of low dose TiO2 resulted in a modest loss of cell density only in the transverse region of the HGS. High dose TiO2, conversely, caused a rapid reduction in cell density as early as 24 hrs following exposure in the proximal vessel. Similarly, population density was also lost in the transverse and distal regions. Furthermore, cell density recovered to the original levels following cessation of TiO2 addition. Microbiota profiling via 16S rRNA gene based high-throughput sequencing did not reveal any specific susceptibilities to TiO2 exposure within individual HGS regions at the Class level. Comparison of the various ratios between bacterial abundance, and assessment of gram status differences during pre- TiO2, and post-exposure periods; indicated a broad effect of TiO2 on the microbiota. We also did not notice a specific change in measures of community diversity, with alpha diversity and evenness maintaining similar values during all measured periods. Predicted functional capacity of the microbiota also remained unchanged during TiO2 exposures. Interestingly, Scanning Transmission Electron Microscopy (STEM) indicated close association of TiO2 particles to bacterial cells, but no direct interaction. These results provide evidence for the negative impact of TiO2 on the whole gut microbiota community independent from the host following recurring exposures.

2192 Impact of Various Surface Coatings on In Vitro Cell Uptake and Cytotoxicity of Ultrasmall Superparamagnetic Iron Oxide Nanoparticles (USPION)


USPION are excellent candidates for medical applications, due to their unique physicochemical properties (e.g., nanoscale size, highly reactive surfaces, and superparamagnetism). However, the potential adverse health effects of USPION with different coatings, commonly employed to control their biological activity and stability, are not fully understood. Therefore, the goal of this study was to evaluate cellular uptake and cytotoxicity of carboxyl- and amino-coated USPION on human coronary artery endothelial cells (HCAEC) as a vascular cell model. Both types of USPION were spherical with average hydrodynamic diameter of ~100 nm as assessed by DLS, and negatively charged (-36.3 and -6.5 mV for carboxyl- and amino-coated USPION, respectively) according to zeta potential analyses. After heat sterilization, the deposited dose. After exposure, cell lines were assessed for cytotoxicity, ROS, and mitochondrial membrane potential (ΔΨm). BEAS-2B demonstrated significant dose-dependent sensitivity to iNECs than incinerated pristine counterparts. In pSAE cells, cytotoxicity, enhancement in ROS, and dissipation of ΔΨm caused by PC, PC-CNT, and PU-CNT were generally lower in magnitude compared to BEAS-2B cells at treatments examined, and is likely attributable to differences in depositional characteristics between the respective culture media for both respective cell lines. Whilst the effect of iNECs on the distal respiratory airway epithelia remains limited in interpretation, the current in vitro model of the respiratory bronchial epithelia demonstrated profound sensitivity to iNECs at depositional doses plausibly relevant for occupational cohorts, indicating potential risk to occupational cohorts with direct exposure to incinerated thermoplastics NECs during disposal.

2193 Incinerated Carbon Nanotube-Enabled Thermoplastics Enhance Cytotoxicity in Human Airway In Vitro Models

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Engineered nanomaterials (NMs) are increasingly being incorporated into synthetic materials as fillers and additives. However, the potential pulmonary exposure effects of NM-enabled composites (NECs) during recycling and disposal have not been adequately addressed. The current investigation aims to characterize the cytotoxicity of incinerated NM-enabled thermoplastic composites (iNECs) on two in vitro pulmonary models. Ultrafine particles released from thermally decomposed pristine PC and carbon nanotube-containing polycarbonate (PC/-CNT) and polyurethane (PU/-CNT) were captured on inline filters, extracted, and suspended in sterile water. Incinerated thermoplastics were ultrasonicated and diluted in culture medium for acute in vitro exposure to primary small airway epithelial (pSAE) and BEAS-2B cells. The Harvard DG model was utilized to estimate the particle settling into the cellular microenvironment to characterize in vitro deposited dose. After exposure, both cell lines were assessed for cytotoxicity, ROS, and mitochondrial membrane potential (ΔΨm). BEAS-2B demonstrated significant dose-dependent sensitivity to iNECs than incinerated pristine counterparts. In pSAE cells, cytotoxicity, enhancement in ROS, and dissipation of ΔΨm caused by PC, PC-CNT, and PU-CNT were generally lower in magnitude compared to BEAS-2B cells at treatments examined, and is likely attributable to differences in depositional characteristics between the respective culture media for both respective cell lines. Whilst the effect of iNECs on the distal respiratory airway epithelia remains limited in interpretation, the current in vitro model of the respiratory bronchial epithelia demonstrated profound sensitivity to iNECs at depositional doses plausibly relevant for occupational cohorts, indicating potential risk to occupational cohorts with direct exposure to incinerated thermoplastics NECs during disposal.

2194 The Influence of Fluid Dynamics on Nanomaterial Delivery and Toxicity: Elucidating the Roles of Particle Size and Cell Model


Colloidal silver nanoparticles (AgNPs) are being increasingly utilized in biomedical applications. However, the effectiveness of these procedures are dependent upon sustained, strong interactions between AgNPs and the surrounding environment. Therefore, prior to the development of effective NP-based therapeutics an accurate means of assessing NP delivery must be established. Both in vitro and in vivo methodologies are being utilized, however, limited correlation exists between these models. One way to overcome this limitation is through the development of enhanced in vitro environments that retain the advantages of cellular systems but more accurately mimic true physiology. In this work, a dynamic in vitro environment was utilized to characterize the AgNP deposition efficiency. Dynamic flow was generated through the use of a peristaltic pump, operating at a flowrate equivalent to known capillary rates. To better understand how dynamic flow impacted deposition, two cell models were utilized; an adherent lung epithelial model (A549) and a suspension monocyte model (U937). Additionally, as bio-transport mechanisms are a function of particle size we included two experimental, citrate-coated AgNPs - 5 and 50 nm. AgNP deposition was evaluated and found to vary as a function of flow environment, cell model, and primary particle size. Dynamic flow significantly decreased the delivered dose of AgNPs to the adherent lung cells; with the 5 nm AgNPs experiencing the greatest drop in deposition. However, AgNP delivery was increased within a dynamic environment for the monocytes, due to increased nano-cellular interactions. For both cell models, the subsequent cytotoxicity, stress, and inflammatory responses correlated to delivered NP dosages, as assessed via reactive oxygen species production, p53 levels, and cytokine secretion. This work highlights the need for NP deposition and safety evaluations to be carried out in a physiologically relevant exposure system.
2195 Label-Free 3D Raman Imaging of Carbon Nanotubes in Mammalian Cells

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Multi-walled carbon nanotubes (MWCNTs) pose a respiratory hazard because they can cause pulmonary fibrosis, which may lead to mesothelioma. The Bionanosciences Group at UT Dallas is interested in understanding the interactions of triloblock polymer Pluronic F-108-coated MWCNTs with macrophages that are the first responders to nanoparticles encountered in the body. This information is relevant for improving the biomedical efficacy of MWCNT therapeutics, for understanding mechanisms of MWCNT biocompatibility, and for developing methods to monitor MWCNT toxicity. To better understand mechanisms of MWCNT toxicity, it is important to know whether MWCNTs physically enter cells and where they locate inside cells. We have developed procedures to detect internalized MWCNTs and reconstruct 3D images of RAW 264.7 mouse macrophages with cell-associated MWCNTs by laser scanning confocal Raman microscopy. Images of cells are reconstructed with stacks of optical sections from confocal planes to place the subcellular MWCNT locations in the context of the intact cell. The 3D MWNT Raman images demonstrated that Pluronic F-108-coated pristine MWCNTs (P-MWNTs) and carboxylated MWNTs (C-MWNTs) exposed to RAW 264.7 cells for 24 h at 37 °C accumulated inside the cells within punctate vesicles, most likely in the endosome/lysosome system, but not in the cytoplasm. These results are consistent with our previous observations that RAW 264.7 cells accumulate ~100 times more C-MWNTs suspended in the surfactant Pluronic F-108 than corresponding P-MWNTs during a 24 h incubation at 37 °C (Wang et al., 2018). Future work will involve assessing the intracellular distributions of various functionalized MWCNTs and evaluating their fate after internalization, and whether they are degraded, secreted, or if they persist within cells. Also, Raman imaging with live cells will be used to access whether accumulation of MWNTs induce the release of cytokines that may cause lysosomal membrane damage and result in the redistribution of MWNTs to the cytoplasm. A better understanding of the mechanisms by which MWNTs interact with macrophages should lead to better rational designs of safer carbon nanomaterials. Wang et al., “Quantitation of cell-associated carbon nanotubes: Selective binding and accumulation of carboxylated carbon nanotubes by macrophages.” Nanotoxicology (2018): 1-22.

2196 Titanium Dioxide Nanoparticle Induced AP-1 Activation via ERKs and p38 Kinase

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Titanium dioxide (TiO2) is a white mineral used in the manufacturing of paint, paper, plastics, sun tan lotion, and other products. Recent studies indicated that TiO2 nanoparticles cause chronic inflammation and lung tumor formation in rats. It is believed that the toxicity and carcinogenesis of TiO2 is associated with particle size. Since activator protein-1 (AP-1) is known to play an important role in the induction of neoplastic transformation and regulation of multiple genes involved in cell proliferation and inflammation, we investigate the potential of TiO2 nanoparticles on reactive oxygen species (ROS) generation and AP-1 signaling in a mouse epithelial cell line, JB6 cells. Incubation of JB6 cells with TiO2 nanoparticles resulted in a dose dependent generation of -OH radicals. TiO2 nanoparticles caused a 3-fold increase in AP-1 activity in the cells. The induction of AP-1 activity in cultured cells was dose-dependent. The signal transduction pathways for AP-1 activation were also investigated and the results demonstrate that TiO2 stimulates mitogen-activated protein kinase (MAPK) family members, including extracellular signal-regulated protein kinases (ERKs), p38 kinase, and C-Jun N-terminal kinase (JNKs). Inhibition of ERKs, p38 kinase, but not JNKs with specific inhibitors SB203580, PD98059, and SP600125, inhibited TiO2 nanoparticles-induced AP-1 activation, respectively. These findings demonstrate that TiO2 nanoparticles stimulate the generation of -OH radicals and induces AP-1 activation, which may be mediated through p38 kinase and ERKs pathways.

2197 Particle Size and Surface Charge Dependent Toxicity of PAMAM Dendrimers in Cultured Endothelial Cells

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Different nanomaterials are under development for various biomedical applications in which nanoparticles contact blood and vasculature. Therefore, investigating the interactions between nanomaterials and vascular endothelial cells is of great importance. Here, we show the effects of polyamidoamine (PAMAM) dendrimers of different two sizes, generation 2 (G2; approximately 3 nm diameter) and generation 7 (G7; 9 nm), with neutral (OH-terminated), anionic (COOH-terminated) and cationic (NH3-terminated) surface modifications on cultured human umbilical vein endothelial cells (HUVECs). HUVECs and extracellular membrane vesicles (EVs) were characterized by flow cytometry (FC), nanoparticle tracking analysis (NTA), laser scanning confocal microscopy (LSCM) and electron microscopy (SEM, TEM). We found that only cationic dendrimers (G2 & G7) induced significant HUVEC apoptosis. G7-NH3 interacted strongly with HUVEC plasma membranes and mitochondrial membranes, and phospholipid vesicles containing G7-NH3, formed, which resulted in extensive plasma membrane blebbing and disintegration. Furthermore, flow cytometric analysis showed that G7-NH3-treated HUVECs released large numbers of extracellular vesicles (EVs) positive for CD105 and PS. A notable population of EVs positive for the mitochondrial marker TOM20 but negative for the autophagosome marker LC3 was found. In summary, large cationic PAMAM dendrimers (G7-NH3) showed both proinflammatory and proapoptotic effects in endothelial cells; at high dendrimer concentrations, these effects were accompanied by necrotic cytotoxicity. G7-NH3 caused plasma and mitochondrial membrane disintegration and the release of EVs, including EVs of mitochondrial origin that were not associated with mitophagy. The findings and conclusions in this study have not been formally disseminated by the US FDA and should not be construed to represent any Agency determination or policy.

2198 Developing a Protocol for Observing the Effects of Sex Differences on Macrophage Polarization

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Macrophages are the primary innate immune cells in the lungs. They protect the lungs by phagocytosing inhaled foreign particles and organisms. Macrophages exhibit several different phenotypes with distinct functions—the “classically activated” M1 phenotype, which is associated with inflammation, or the “alternatively activated” M2s, which can be divided into a variety of subsets. There is evidence that estrogen receptor alpha (ERα) signaling promotes M2 macrophage development, contributing to sex differences seen in human respiratory disease prevalence. For example, it may help explain why asthma, an M2-driven disease, occurs more often in women than in men. However, the exact role that sex hormones play in respiratory pathophysiology is still unknown. Understanding how sex hormones influence macrophage phenotype development in the lungs will help us address this gap of knowledge. The goal of this project was to develop a protocol for macrophage phenotype and ERα expression analysis via flow cytometry. Developing an effective protocol for cytometric assessment of these proteins is challenging due to our need to simultaneously assess both cytoplasmic and nuclear proteins, as well as extracellular markers. Murine bone marrow-derived macrophages and alveolar macrophages (AMs) were polarized into M1 and M2 pheno- types and analyzed for ERα, M1 markers CD38 and Ly6C, and the M2 marker YM1. After establishing that our fixation and permeabilization protocol was suited for all markers of interest, we determined effective concentrations for all markers of interest, we determined effective concentrations for all markers of interest, we determined effective concentrations for all markers of interest. Expression of the M1 and M2 phenotype markers corresponded appropriately to the M1- and M2-polarized macrophages, respectively. ELISA measurement of cytokines was used to corroborate our cytometric results. Overall, this project improved our protocols for studying macrophages in order to better understand how sex hormones affect macrophage phenotype development. In the future, we will apply these protocols to in vivo studies assessing sex differences in the immune response to inhaled particles. This project was supported by NIH grants R25 ES022866 and R01 ES023209.

2199 Evaluating the Toxicity of Silver Nanoparticles in ARPE-19 Cells through High-Content Morphometric Analysis


Efficient methods are needed to evaluate the human and environmental health effects of silver nanoparticle (AgNP) exposure. A high content imaging-based phenotypic profiling approach was used to determine the effects
of 12 types (40, 60, 80 and 100 nm coated with citrate, polyvinylpyrrolidone (PVP), and branched polyethyleneimine (BPEI)) of AgNPs on organelle morphology in human retinal pigmented epithelial (ARPE-19) cells. AgNPs (0.1-30 µg/ml) were applied to the cells seeded in a 384-well format, with silver nitrate acting as silver ionization control. The Distorted Grid (DG) model was used to estimate the cellular delivered dose. After 24 hrs of treatment, cells were live-labeled with MitoTracker (mitochondria), fixed, permeabilized and labeled with Hoechst-33342 (nuclei), SYTO14 (nucleoli) and fluorescent conjugates of concanavalin A (ER), phalloidin (actin cytoskeleton), and wheat germ agglutinin (Golgi/plasma membrane). A multiplexed cell viability and apoptosis assay was run in parallel. Cells were imaged using an Opera Phenix High Content Screening System and profiled using Harmony High Content Analysis software. Approximately 1200 morphological features were measured per cell and summarized to the well level for analysis. The DG model predicted that the fraction of AgNP deposited on cells increased with particle size and differed based on coating (BPEI > citrate > PVP). Phenotypic profiling showed that all AgNP types affected the organelle morphology in a concentration-dependent manner, with over 600 features having benchmark doses below the threshold for cytotoxicity. The pattern of changes in cell morphology differed across coating agents and silver nitrate. Citrate coated AgNPs showed the most pronounced effects below the cytotoxic threshold. 60 nm PVP had fewer effects on cell morphology than other coatings, yet apoptosis was observed at an estimated delivered dose as low as 1.45 µg/ml. This screening method may inform subsequent assay selection by highlighting the intracellular regions affected by AgNPs of interest. This abstract does not necessarily reflect US EPA policy.

Potential Role of Thioredoxin-Interacting Protein in Silver Nanoparticle-Mediated Mast Cell Degranulation

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Silver nanoparticles (AgNPs) are being incorporated into a variety of consumer and medical products primarily due to their antimicrobial properties. This will lead to a significant rise in exposure to the general population; however, our understanding of the potential risks is still minimal. We have previously demonstrated that AgNPs induce a non-IgE mediated degranulation of mast cells. Importantly, we have also shown a strong genetic contribution to AgNP driven mast cell degranulation. RNA sequencing performed on bone marrow-derived mast cells (BMMCs) from high-responding (C57BL/6) and low-responding (LP/J) strains of mice demonstrated a significant increase in thioredoxin-interacting protein (txnip) in the low-responding strain after exposure to 20nm AgNP. We therefore explored the role of TXNIP in mast cells to determine its potential regulatory role in AgNP-mediated degranulation. The following exposure to AgNP, txnip mRNA levels were increased in LP/J BMMCs at six hours post-exposure while protein levels were significantly decreased in C57BL/6 BMMCs. siRNA knockdown of TXNIP resulted in a trend towards increased degranulation. Using a Seahorse XF analyzer, we found that BMMCs from low- and high-responding strains possess varying glycolytic capacities in response to AgNP exposure possibly implicating TXNIP as a regulatory protein for cellular metabolism during mast cell degranulation. Our data suggests that TXNIP plays a role in non-IgE mediated mast cell degranulation initiated by exposure to 20nm AgNP and may possibly modulate cellular metabolism.

Amorphous Silica Coating Protects against Iron Oxide Nanoparticle-Induced Cell Transformation and Genotoxicity

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Iron oxide nanoparticles (IONP) have a wide range of uses in biotechnology, medicine, and transportation. However, very little is known about their potential adverse health effects following human exposure. Some evidence suggests that dissolution of IONP following endocytosis into cells may disrupt iron homeostasis, resulting in genotoxicity and neoplastic-like cellular transformation. Surface modification of IONP, such as an amorphous silica coating, may impact subsequent adverse outcomes by reducing particle dissolution. The main objective of this study was to assess IONP low dose, long term exposure effects, including carcinogenic potential, as well as the utility of an amorphous silica coating in reducing or preventing these outcomes. Human bronchial epithelial cells (Beas2B) were continuously exposed to nFeO3 or nano-SiO2 coated nFeO3 (SiO2 : nFeO3) for up to 6.5 months at an occupationally relevant low dose (0.65 µg/cm2 or 2.88 µg/ml) and evaluated over time for indications of neoplastic-like transformation and its underlying mechanism. Transformation was compared to that induced by gas metal arc mild steel welding fumes (GMA-WF), which were recently re-classified as a Group 1 total human carcinogen and are comprised of roughly 80% iron/iron oxide. Our results showed that beginning at four months, nFeO3-exposed Beas2B underwent neoplastic-like transformation, as indicated by increased cell proliferation and attachment-independent colony formation. These outcomes correlated with nFeO3 dissolution, increased intracellular iron, and genotoxicity, as well as significant changes in pathways related to DNA damage repair and autophagic processes. nFeO3-induced transformation also closely matched that of GMA-WF induced transformation SiO2 : nFeO3 treatment, however, did not induce any changes in the above parameters. Overall, our results indicated potential carcinogenic risk of nFeO3 associated with particle dissolution, iron homeostasis disruption, and changes in autophagy and DNA damage repair pathways, which were reduced with an amorphous silica surface coating. This study shows the potential utility of a “safe by design” hazard reduction strategy, to alter particle physicochemical properties based on mode of toxicity to reduce risk.

In Vitro Dermal Toxicity of Redox-Active Metal Nanocatalysts

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Nanocatalysts (NCT) represent the convergence of catalysts, a mature technology with a new one, nanotechnology. NCT is a rapidly growing field that involves the use of nanomaterials as catalysts for a variety of catalytic applications. Since metal nanoparticles (MeNPs) have a large surface-to-volume ratio compared to bulk materials, they are attractive candidates for use as catalysts. A number of redox-active MeNPs and their oxides (MeO) including nickel (Ni) and cobalt (Co) are widely used. The physical nature and reactive surface properties of some of these may affect their ability to induce dermal toxicity thus causing adverse skin reactions. We hypothesize that toxicity of Me/MeO NP occurs via their ability to initiate oxidative stress, thereby inducing redox-sensitive transcription factors and triggering inflammation. Moreover, due to the skin’s susceptibility to UV radiation, it is important to evaluate whether Me/MeO NP enhance the adverse effects of UVB. To test these hypotheses, the effects of Ni, Co, NiO, CoO, Co3O4 and CoO alone (0-26 µg/cm2) and co-exposed with UVB (4KJ/m²) were studied in vitro and in situ using murine epidermal cells (B6P+) and an engineered human skin construct (EpiDerm FT). Cell exposure to Me/MeO NP resulted in a dose- and time-dependent loss of cell viability, cell damage, oxidative stress and activation of AP-1/NF-kB. Co-exposure of Me/MeO NP with UVB ensued in amplification of the observed effects. Exposure of EpiDerm FT to Me/MeO NP caused tissue damage, oxidative stress and accumulation of inflammatory mediators. Hierarchical cluster analysis resulted in two major clusters separating cytokines productions related to inflammatory cell recruitment (more intense) and T2-type/ regulatory immune responses (dimmed). UVB exposure alone induced significant tissue damage and secretion of cytokines/chemokines. Ni compounds drastically enhanced the percutaneous LDH and UVB across the whole cytokine spectrum, while Co oxides prompted much weaker reaction. Interestingly, inflammatory cytokine/chemokine levels upon exposure to Me/MeO NPs, with or without UVB pre-treatment, followed similar trends compared to cell/tissue damage i.e., NiO > NiO3-CoO > CoO and correlated with their similar effects on UVB in both in vitro and in vivo settings. Ligation of human and the adverse effects of Me/MeO NP could induce cytotoxicity, oxidative stress and inflammation, and may potentially enhance response caused by UVB pre-treatment. Disclaimer: The findings and conclusions of this report are those of the authors and do not necessarily reflect those of National Institute for Occupational Safety and Health.

 Comparative In Vitro Study of Adverse Pro-neoplastic Potential of Tremolite Asbestos and its Cleaveage Fragments in Human Epithelial (BEAS-2B) and Mesothelial (MET-5A) Cells

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The fibrous form of tremolite is one of the six recognized types of asbestos. It is known that inhalation of respirable tremolite fibers (TF) can cause asbestosis, lung cancer and both pleural and peritoneal mesothelioma. Tremolite also occurs in a non-fibrous habitat that can be mechanically broken into cleavage fragments (CF) which can meet the criteria for fibers. Despite the considerable amount of work showing that CF are less potent in their carcinogenic effects compared to TF, they may play a role in the process of mesothelioma development. Using a Seahorse XF analyzer, we found that CFs have a significant impact on cell metabolism and growth while not inducing cell death. Using a Seahorse Galaxy platform, we found that CFs induce a strong metabolic shift in MET-5A cells including decreased metabolism and increased oxidative stress. These results coupled with the finding that CFs are present in the lungs of patients diagnosed with mesothelioma indicate that CFs may be a potential factor in the etiology of this malignancy independently of the TF exposure. These findings and implications may prove helpful for future research.
ing CF (with similar median length but different width/aspect ratio) using in vitro models. Sub-chronic exposures of human epithelial (BEAS-2B) and mesothelial (MET-5A) cells - both target cells of the respective lung cancer and malignant mesothelioma - to TF and CF were employed in the current study. Cells were evaluated for the presence of several cancer hallmarks indicating the neoplastic transformation following continuous exposure to the sub-toxic concentration of TF and CF for 5 weeks. TF-exposed cells, both BEAS-2B and MET-5A, revealed a neoplastic-like transformation phenotype characterized by significant increase in proliferation, morphological transformation, invasion/migration and anchorage-independent growth compared to controls. In contrast, no anchorage-independent growth was observed in CF-exposed cells although an increase in proliferation, morphological transformation and migration/invasion was detected albeit at lower intensity compared to TF. Additionally, CF had no impact on apoptosis susceptibility while TF caused increased apoptosis in MET-5A cells and its inhibition in BEAS-2B cells. Both TF and CF induced oxidative DNA damage albeit with a stronger effect in TF-exposed cells. Analysis of inflammatory responses using a cytokines/chemokines clustering approach suggested cell-type specific effects to TF and CF exposures as well as treatment related differences. Overall, our data are compatible with the interpretation that tremolite asbestos fibers demonstrated higher neoplastic transformation potential compared to CF (at the same mass dose) in both epithelial and mesothelial cells. Disclaimer: The findings and conclusions of this report are those of the authors and do not necessarily reflect those of National Institute for Occupational Safety and Health.

2204 Effects of Multiwalled Carbon Nanotube Accumulation on Macrophage Cell Viability and Proliferation In Vitro

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The range of applications for multi-walled carbon nanotubes (MWNTs), from electronics to medicine, is increasing their global production despite an incomplete understanding of their toxicological potential. A recent study reported that more than 99% of all nanoparticles administered in vivo are sequestered by macrophages, but the types and severity of effects induced by MWNT accumulation in macrophage cells is not well understood. Our previous work showed that macrophages preferentially accumulate a~100X more Pluronic® F108-coated carboxylated MWNTs (cMWNTs) than non-functionalized pristine MWNTs (pMWNTs) (Wang et. al., Nanotoxicology, 2018, DOI: 10.1080/17435390.2018.1472309). Furthermore, cMWNT accumulation in RAW 264.7 cells after a 20h exposure impaired subsequent phagocytic function. Our recent work focuses on the effects of cMWNTs on other macrophage cell functions, specifically cell proliferation and viability. To assess cell proliferation, RAW 264.7 cells were treated with varying concentrations of cMWNTs or pMWNTs and incubated at 37°C for 24, 48, or 72 hours. The cells were then washed to remove MWNTs in the media and proliferation was measured with a crystal violet assay. The results showed that neither cMWNT nor pMWNT accumulation significantly affected cell proliferation after a 24h exposure, but cell proliferation was reduced by as much as 88% and 95% after exposure to cMWNTs at 200 µg/mL for 48h and 72h, respectively. The severity by which cell proliferation decreased between 24h and 72h of exposure raised the question of whether 24h exposure to cMWNTs affected cell viability in ways undetected by the cell proliferation assay. Consequently, colony formation assays were conducted in which RAW 264.7 cells were treated either with pMWNTs at 100 or 200µg/mL, or with cMWNTs at concentrations ranging from 25 to 200µg/mL and incubated at 37°C for 24h. The cells were then washed, harvested, seeded at a density of 1000 cells per plate, and incubated at 37°C in media free of MWNTs for 10 days, after which the colonies were stained and counted. There were 371 ± 22, 248 ± 27, and 187 ± 13 colonies per plate for the control, pMWNT-treated, and cMWNT-treated cells, respectively, which suggested that cMWNT accumulation over 24h impaired cell viability by 49.6%. Additional experiments will explore the effects of cMWNTs on reactive oxygen species and apoptosis.

2205 Evaluation of Cytotoxicity Potential of 6-Thioguanine Loaded Chitosan Nanoparticles with or without Curcumin


CANCER is the second leading cause of mortality in the world. Cancer nanotherapeutics are rapidly progressing and being implemented to overcome several limitations of conventional drug delivery systems. The objective of the study was to synthesize 6-thioguanine (6-TG) loaded chitosan nanoparticles (CNPs) and to evaluate the cytotoxicity with or without curcumin (CUR) on two cancer cell lines viz. Breast adenocarcinoma (MCF-7) and Ovarian teratocarcinoma (PA-1). 6-TG loaded CNPs were formulated by ionic-gelation method. Morphologically the 6-TG loaded CNPs were spherical in shape and showed mean size, PDI, zeta potential and Entrapment efficiency of 261.63 ± 6.01 nm, 0.35 ± 0.10, 15.97 ± 0.46 mV and 44.27% respectively. MTT [3-(4, 5- dimethylthiazolyl)-2]-2, 5- diphenyltetrazolium bromide] assay was conducted on MCF-7 and PA-1 cell lines at 48 h incubation for cytotoxic evaluation. IC₅₀ values of 6-TG, 6-TG loaded CNPs and CUR for MCF-7 were 23.09 µM, 17.82 µM, 11.48 µM and 15.73 µM respectively. Likewise, IC₅₀ values of 6-TG, 6-TG loaded CNPs and CUR for PA-1 were 5.81 µM, 3.92 µM and 12.89 µM respectively. Combination of 6-TG loaded CNPs (IC₅₀) with CUR (IC₅₀) on PA-1 and MCF-7 showed percent cell viability of 43.67 ± 0.02 and 49.77 ± 0.05 respectively. This study suggests that the cytotoxicity of 6-TG and 6-TG loaded CNPs is dose-dependent and 6-TG loaded CNPs proved to be significantly more effective (~1.5-fold high anticanicancer efficacy) than that of 6-TG against PA-1 cells. Further, combination of 6-TG loaded CNPs with CUR showed synergistic cytotoxicity i.e. enhanced anticancer efficacy on PA-1 cancer cells.

2206 Bioactivity of Multiwalled Carbon Nanotube Mixtures with Multiple Aspect Ratios

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Multi-walled carbon nanotube (MWCNT) composites have vastly superior mechanical and structural properties compared to conventional materials. In order to be cost effective and to improve the distribution in the composite matrix, MWCNTs produced in large volume with multi-aspect ratios are utilized. Past research identified pulmonary health effects and the molecular mechanisms associated with exposure to mostly uniformed dimensional tubes; however, much is unknown concerning exposure associated with mixtures containing multi-aspect ratio tubes. In order to investigate this concern, we evaluated the toxicity profile of two multi-aspect-ratio MWCNT mixtures (MWCNT-1 and MWCNT-2) and compared them with the toxicity profile of more uniform and well-characterized MWCNT (Mitsui-7) and carbon black (CB, Printex-90) samples. Automated field emission scanning electron microscopic analysis showed that the MWCNT mixtures had a wide distribution of lengths from a few nanometers up to 20 µm and diameters that change according to length. The Mitsui-7 were more uniform with a diameter ~50 nm and the CB had a diameter of ~20 nm. Cytotoxicity and cell proliferation was assessed in human monocytic cells (THP-1) at 0 - 120 µg/mL and in primary human fibroblast cells (PHF) at 0 - 16 µg/mL NFkB (nuclear factor kappa-light-chain-enhancer of activated B cells)-induced inflammatory potential was screened using THP-1 reporter cells. THP-1 WT and NLRP3-deficient cells were used to screen for inflammasome activation. Acellular oxidative stress potential of the material correlated with the fold increase in vitro NFkB activation and oxidative stress induction, measured using a dichlorofluorescin-diacetate assay. The extracellular remodeling and fibroblast transformation potential was evaluated by measuring collagen I, α-smooth muscle actin, and TGF-B in PHF cells. Given the toxicity and the metric for molecular initiating events, the MWCNT-2 mixture was the most bioactive material followed by Mitsui-7 > CB > MWCNT-1. We conclude that it was not possible to fit all multi-aspect-ratio MWCNTs into a universal toxicity profile. Ongoing extensive physiochemical characterization could elucidate the key confounders influencing the toxicity profiles of such a multi-aspect-ratio MWCNT mixture.
Silver nanoparticles (AgNP) are found in a range of applications but are primarily used as antimicrobial agents in consumer and medical products. Many studies have assessed the in vitro and in vivo effects of AgNP using airway models, but none have directly studied how their impacts are modified by individual differences in genetic and acquired susceptibility, or gene x environment interactions (GxE). In this study, we derived airway epithelium organotypic culture models from murine tracheal epithelial cells to assess determinants of susceptibility to AgNP toxicity. We compared dosimetrically adjusted dose-response relationships for AgNP on barrier dysfunction, glutathione (GSH) depletion, reactive oxygen species (ROS) production, lipid peroxidation, and cytotoxicity across genetic strains (A/J and C57BL/6), differentiation conditions (normal and “Th2-skewed” conditions) and several exposure durations (a single exposure of 24 hours and a repeated exposure of 4 hours, every other day, over 5 days (5×4 hours)). In this study, we found that organotypic cultures differentiated under “Th2-skewed” conditions are a model for allergic airway diseases, such as asthma. Using a benchmark dose (BMD) approach, we found the most sensitive adverse cellular responses to be ROS production and lipid peroxidation at 5×4 hours conditions in organotypic cultures differentiated under “Th2-skewed” conditions. This study highlights the importance of using multiple genetic strains and differentiation conditions to identify determinants of susceptibility. Such in vitro assessments can be used to inform regulatory policy aimed at special protections for susceptible populations.

Engineered nanomaterials (ENM) are becoming increasingly prevalent, both in consumer products and in medical applications. The proliferation of this technology increases the likelihood of human respiratory exposure to ENM. Studies have shown that ENMs can induce permeabilization of lipid membranes in cells. For example, when ENMs are phagocytosed by macrophages, ENMs can interact with phagolysosome membranes. These ENMs can destabilize these membranes, resulting in the release of cathepsin B from the phagolysosome and subsequent interleukin 1β release. Lanthanum titania oxide (LTO) is of interest because of its wide spread use in electronics, magnets, and batteries. The objective of this work was to evaluate the behavior of pristine (pLTO) and N-doped (nLTO) nanoparticles in macrophages and the mechanism of particle-induced lysosomal membrane permeabilization (LMP) using multiple membrane models. It was hypothesized that pLTO would cause membrane disruption by increasing lipid order, creating tighter packing and decreased membrane fluidity. Bone marrow derived macrophages (BMDM) were used for LMP assay. Human red blood cell (RBCs) and liposomes (DOPS and POPC) were used as a simplified surrogate for internal cellular membranes. RBCs and liposomes were exposed to the LTO particles and changes in membrane order were measured using fluorescence lifetime and anisotropy on a custom-built fluorometer with the fluorescence probe Di-4-ANEPPDHQ. Additionally, ENM-induced membrane leakage in RBCs was measured using a hemolysis assay. We saw greater LMP from pLTO exposed A549 cells than from nLTO exposed A549 cells. It was hypothesized that pLTO-exposed nanoceria, attributed to C=O groups from milk components, and suggestion of loss of, or overcoating of, citrate after exposure to each SBF. FeGF exposure increased the isoelectric point from pH 2.5 (citrate-coated nanoceria) to 3.0. Exposure to the other SBFs had less effect. Surface charge was more negative after exposure to all SBFs, except FeGF. A549 cells were incubated for 2 h with SLF and Caco-2 cells with 75% FaGF or FeGF:25% cell culture medium or 90% FaIF or FeIF:10% cell culture medium containing 0, 1, 5, 20, or 100 µg/cm² SBF exposed nanoceria. SBF dilution with cell culture medium was necessary because cells did not survive exposure to these undiluted SBFs. The resazurin assay quantified cell metabolism. Exposure to SBFs below 100 mcg/cm² non-significantly increased cell metabolism in many cases. A decrease of cell metabolism (non-significant) was only seen after exposure to 100 mcg/cm² FaGF, FeGF, and FeIF. The increased hydrodynamic size (that would reduce uptake/absorption) and lack of reduced cell metabolism below 100 mcg/cm² (orders of magnitude above the reported environmental aerosol concentration), suggest SBF-induced corona formation does not increase nanoceria adverse effect risk. Support: NSF REU EEC-14604861 and NIH R01GM101995.

Mitochondria have several important functions in eukaryotic cells including ATP production, reactive oxygen species regulation, and programmed cell death. These functions are intimately tied to mitochondrial structure as dysfunctional mitochondria often display structural defects. Given how important mitochondria are to cell health, it is surprising that mitochondrial structure and function are understudied areas in nanotoxicology. Silver nanoparticles (AgNPs) have antibacterial properties and have been incorporated into unilamellar liposomes (DOPS and POPC) have antibacterial properties and have been incorporated into unilamellar liposomes. For example, AgNP coated liposomes were found to be more toxic than pristine liposomes. The objective was to determine if simulated body fluid (SBF) exposure changes its surface properties and toxicity. Crystalline citrate-coated nanoceria (primary particles ~30 nm) was incubated with simulated lung (SLF) for 3 h, fasting- (FaGF) or fed-gastric (FeGF) for 2 h, and fasting- (FaIF) or fed-intestinal fluid (FeIF) for 6 h, water washed, and dried. Exposure to each SBF increased the hydrodynamic diameter. Thermogravimetric analysis indicated organic coating on the FeGF- and (to lesser extent) FeF-exposed nanoceria. FTIR showed organic coating on the FeGF-exposed nanoceria, attributed to C=O groups from milk components, and suggestion of loss of, or overcoating of, citrate after exposure to each SBF. FaGF exposure increased the isoelectric point from pH 2.5 (citrate-coated nanoceria) to 3.0. Exposure to the other SBFs had less effect. Surface charge was more negative after exposure to all SBFs, except FeGF. A549 cells were incubated for 2 h with SLF and Caco-2 cells with 75% FaGF or FeGF:25% cell culture medium or 90% FaIF or FeIF:10% cell culture medium containing 0, 1, 5, 20, or 100 µg/cm² SBF exposed nanoceria. SBF dilution with cell culture medium was necessary because cells did not survive exposure to these undiluted SBFs. The resazurin assay quantified cell metabolism. Exposure to SBFs below 100 mcg/cm² non-significantly increased cell metabolism in many cases. A decrease of cell metabolism (non-significant) was only seen after exposure to 100 mcg/cm² FaGF, FeGF, and FeIF. The increased hydrodynamic size (that would reduce uptake/absorption) and lack of reduced cell metabolism below 100 mcg/cm² (orders of magnitude above the reported environmental aerosol concentration), suggest SBF-induced corona formation does not increase nanoceria adverse effect risk. Support: NSF REU EEC-14604861 and NIH R01GM101995.
2212 Effect of Particle Size on the Cytotoxicity of Zinc Oxide Nanoparticles in Rat and Human Intestinal Cell Models


Zinc oxide nanoparticles (NPs) have several commercial applications ranging from catalysts to semiconductors. ZnO NPs are toxic to bacteria, aquatic organisms and human cells. There is a potential for human oral exposure to ZnO NPs following accidental or intentional ingestion, hand-to-mouth activity, or mucociliary transport following inhalation. This study assessed the cytotoxic effects of ZnO NPs (10 and 150 nm) in rat and human intestinal cells. The rat cells are a 2-dimensional model (IEC-6) while the human cells are a 3-dimensional highly differentiated model. Three-dimensional cell cultures offer greater predictability of in vivo toxicity than comparable 2-dimensional cell cultures because of their complexity and their overall functions are more similar to native tissues. The effect of dose (0.1 - 100 μg/mL rat; 1-100 μg/mL human), time (4 and 24 hr) and particle size were evaluated. ZnSO₄ (0.1-100 μg Zn/mL) was tested for 4 and 24 hr to assess Zn ion toxicity. IEC-6 cells were plated at 60k/well in a 96 well plate 24 hr before dosing. Media with 10% fetal bovine serum was the negative control. Triton X-100 (0.3%) was the positive control. ZnO NPs were suspended in media and probe sonicated before dosing. Following incubation, the cells were washed with media. Cytotoxicity was assessed using a colorimetric method that measures mitochondrial activity (MTS; rat; MTT, human). In IEC-6 cells, a significant dose-dependent (p<0.0001) decrease in viability was observed after 4- and 24-hr incubation of the 10 and 130 nm ZnO NPs. For both particles in IEC-6 cells, at 4 hr, viability decreased ~50% at ≥ 50 μg/mL; at 24 hr, viability decreased ~75% at ≥ 25 μg/mL. In human cells, viability was significantly decreased, but no greater than 10% at 4 or 24 hr, for both ZnO NPs. For ZnSO₄, similar results to the ZnO NPs were observed in both tissues at 4 and 24 hr. Particle size does not appear to have a role in the cytotoxicity of ZnO NPs in these cells. In addition, the similar cytotoxicity profile of ZnO NPs and Zn²⁺ ions suggests dissolution of the NPs may have a greater impact than particle size. This abstract does not necessarily represent US EPA policy.

2213 Effects of Surface Modification of Graphene Quantum Dots on Differentiated THP-1 Human Macrophages


Due to their unique chemical and physical properties, graphene quantum dots (GQDs; <10 nm in diam) are attractive for biomedical applications such as bio-imaging and drug delivery. Previous studies show that bare GQDs cause DNA damage and significantly increase the expression of proinflammatory cytokines (including TNF-α and IL-8) in macrophages at concentrations where no significant toxicity is observed (<50 μg/mL). However, these studies have been limited to bare GQDs without taking surface modification effects into consideration, which has the potential to passivate the GQDs surface and minimize toxicity. The objective of this study was to investigate the effects of bare, carboxyl- and amino-coated GQDs on differentiated THP-1 human macrophages. Nanoparticle size distribution was assessed by transmission electron microscopy, atomic force microscopy, and dynamic light scattering in water and cell culture medium. GQDs exhibited an average diameter (TEM) of 5-10 nm and surface charges of -22 mV and +19 mV for carboxyl- and amine-coated GQDs, respectively. Aggregation was observed when GQDs were dispersed in the serum-rich cell culture medium, which is probably associated with protein corona formation. Cells were exposed to bare, carboxyl-, and amino-coated GQDs at concentrations of 15, 100, or 250 μg/mL for 24 and 48 hr. No significant cytotoxicity was observed for bare and carboxyl-coated GQDs at all concentrations. For amino-coated particles, a decrease of 40% in cell viability was observed at concentrations >100 μg/mL after 48 hr. The cytotoxicity pathway (e.g., apoptosis vs. necrosis) and release of cytokines were also assessed by flow cytometry and ELISA, respectively. Our findings indicate that the surface coating of GQDs significantly affects particle uptake and toxicity in human macrophages. Further studies are needed to build the toxicological profile of GQDs before use in biomedical applications.

2214 Molecular Toxicity Analysis of Citrate Gold Nanoparticle-Treated Human Intestinal CaCo-2 Cells by Array Gene Expression Profiling

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Some in vitro studies have shown citrate gold nanoparticles (cAuNPs) to be safe for nanomedicine applications (not toxic) via viability assay, while some have shown that toxicity is size-dependent. Meanwhile, in a previous study (Sibuyi et al., 2017), the application of peptide functionalised cAuNPs was established for selective induction of apoptosis in CaCo-2 cells. Unfunctionalized cAuNPs were shown to accumulate inside the cells with no discernible toxicity. However, very little is known about how the uptake of cAuNPs may affect gene expression patterns within cells. Any adverse effects of cAuNPs on gene expression may hamper the use of AuNP in nanomedicine. Consequently, this study aimed to investigate the effects of cAuNPs on gene expression in CaCo-2 cells. Monodisperse, 14 nm spherical cAuNPs were synthesised using the Turkevich method. CaCo-2 cells were treated for 24 hrs with 12.5 nM cAuNPs. The viability and uptake of cAuNPs in CaCo-2 cells were monitored using WST-1 assay and ICP-OES, respectively, and the expression levels of a panel of 84 genes that are related to molecular toxicity were evaluated using quantitative RT-PCR and the RT2 Profiler PCR array. Although the WST-1 assay shows no toxicity of cAuNPs to CaCo-2 cells, gene expression profiling revealed that internalisation of cAuNPs affects the expression of several genes associated with endoplasmic reticulum stress, DNA damage and repair, immunotoxicity, oxidative stress and antioxidant and mitochondrial energy metabolism pathways.

2215 Evaluation of the Skin Sensitizing Potential of Gold Nanomaterials and the Impact of Established Dermal Sensitivity to Gold on the Pulmonary Immune Response with Respect to Dose Mass and Surface Area

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The easily-manipulated physico-chemical properties of gold nanomaterials (AuNM) have proven useful in many biomedical applications. However, the historical use of gold for the treatment of rheumatoid arthritis and the known sensitizing capacity of gold salts suggest that AuNM may exhibit immuno-modulatory properties. To address this, three studies were performed using female C57BL/6 mice. First, the skin sensitizing capacity of different forms of gold was assessed in the Local Lymph Node Assay (LLNA) using soluble gold salt (AuCl₃, 10%) and increasing concentrations (2.5, 5, 10%) of gold particles (Au, 12.1 um) and spherical AuNM (30 nm). Next, the pulmonary immune effects of AuNM (10, 30, or 90 ug) were assessed 1 d, 4 d, and 8 d post-aspiration. Finally, the impact of existing dermal sensitivity to gold on the pulmonary immune response to different forms of gold was assessed. Mice were dermally sensitized to gold by three dermal exposures (1 d, 2 d, 3 d) to 10% AuCl₃ or vehicle control (VC). Mice were then aspirated once (10 d), twice (10 and 14 d), or three times (10, 14, and 18 d) with VC, 30 μg Au, or a dose of AuNM normalized to the mass or surface area of the 30 μg Au (30 μg Au or 0.2 μg, respectively) and euthanized 1 d later. In the LLNA study, AuCl₃ had a stimulation index (SI) of 10.9, in accordance with its known potent sensitizing capacity. Although the SI of AuNM (2.3) was higher than that of Au (1.1), a three-fold increase in lymphocyte proliferation was not observed for either particle, indicating minimal risk for dermal sensitization. In the dose response study, AuNM induced only minimal lung injury and inflammation. However, exposure to the 90 ug dose did induce a significant increase in total number and percent activated CD4⁺+ T-cells and B-cells in the mediastinal lymph nodes at 4 and 8 d. In the alergy study, after two and three aspirations, mice sensitized to gold exhibited elevated lung lymphocyte numbers which correlated to dose surface area. Moreover, serum IgE levels were significantly increased only in dermally-sensitized mice aspirated with AuNM, irrespective of dose, indicating a potential for increased susceptibility to the development of an IgE-mediated adaptive immune response following respiratory exposure. Collectively, the results from these studies suggest existing dermal sensitivity to gold may exacerbate the pulmonary immune effects resulting from AuNM inhalation.
2216 Gender-Specific Biological Responses in Juvenile Rats Orally Exposed to Three Engineered Nanomaterials

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Engineered nanomaterials (ENMs) are widely used in medicine, food, and agriculture, as well as general household applications, and exposure to them is ubiquitous. Children represent a vulnerable population because perturbations in cell growth and signaling can disrupt temporally-sequenced developmental processes leading to long-term functional deficits. Little is known about the behavior, and biological responses of ENMs and their toxicity in developing animals. In this study three nanoparticles (NPs) provided by The NIHEHS Consortium for Nanotechnology Health Implications Research were tested: TiO2, P25, 30 nm Al2O3, and 50 nm ZnO. Three litters (five males and five females) of juvenile Sprague-Dawley rats were daily administered 10 mg/kg NP or vehicle control (water) by oral gavage between postnatal day (PND) 17-20. Basic neurobehavioral (acoustic startle response, locomotor activity, and rotarod) and cardiac (ECGenie) assessment were performed 4 hours post administration of the final dose. Animals were sacrificed on PND 21, and selected tissues were collected, weighted, and processed for histopathology or biochemical analysis. Neurotransmitter concentrations in brain tissues were quantified by HPLC with electrochemical detection. No change was observed for body weight (b.w.) or brain-to-b.w. ratio for pups. Liver-to-b.w. ratio were significantly increased for male pups receiving TiO2 (0.0417±0.0028) and Al2O3 NP and (0.0409±0.0021) and for female pups administered TiO2, P25 (0.0420±0.0040) compared to control (male: 0.0389±0.0025; female: 0.0393±0.0021). No neurobehavioral effects were found. Heart rate was significantly decreased for female pups administered TiO2, P25 (441±43.3 beats per minute (bpm)) compared to vehicle control (512±46.0 bpm). No significant changes were observed for neurotransmitter levels in brain tissue. Enhanced Darkfield and Hyperspectral imaging (CytoViva) are being used to evaluate the presence of NPs in tissue sections of the intestine, liver, spleen, kidney, and lymph nodes. The microscopy analysis is in progress, we have located Al2O3 in the liver. Gender-specific responses were observed in juvenile rats orally administered TiO2, P25 and Al2O3 NP. These data suggest that the developing animal represents a valuable model for oral ENM exposure.

2217 Long-Term Effects of Inhaled Nanoparticles in Rats: Ceriumdioxide and Bariumsulphate

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Ceriumdioxide (NM212) and bariumsulphate (NM220) nanoparticles were tested according to the OECD test guideline no. 453; additions were made to the standard protocol to find evidence of inflammation and potential lung tumours with high sensitivity. Aerosol concentrations were concentrations 0.1, 0.3, 1 and 3 mg/mL and 50 mg/m³ was tested, respectively. Levels of cerium measured in the organs increased with higher exposure concentrations and over time. However, the accumulation only reached a very low level. Lung burdens of barium were unexpectedly low during the first three months of exposure, due to fast clearance most probably by dissolution measured in the organs increased with higher exposure concentrations and tested according to the OECD test guideline no. 453; additions were made to H. Ernst the Federal Institute for Risk Assessment. Toxiology and Experimental Medicine and the biodistribution was analysed by the Federal Institute for Risk Assessment.

2218 Comparative In Vivo Assessment of Alveolar Fibrosis, Histopathology, and Systemic Translocation Induced by Carbon Nanotubes and Nanofibers from US Facilities

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Pulmonary exposure to carbon nanotubes or nanofibers (CNT/F) is known to induce inflammation, toxicity, or tumorigenesis, and is a concern in the occupational setting. CNT/F represent a large class of materials and it is unclear if all confer similar toxicity. Our aim was to use ENM-TED to test the pulmonary effects induced by CNT/F with variable physicochemical properties obtained from U.S. facilities. Seven different multi-walled CNT and two CNF were characterized and evaluated for alveolar fibrosis, translocation, and histopathological changes in mice at 1, 7, 28, and 84 d following oropharyngeal aspiration to 4 or 40 µg of each material. Lung sections at 84 d post-exposure to 40 µg were microscopically evaluated to measure changes in histopathology. Moderate and multifocal granulomatous bronchopneumonia, bronchiolitis obliterans, bronchiolar epithelial hypertrophy, and peribronchiolar fibrosis were observed in most, but not all, high dose exposures. Variances in incidence and severity between the CNT/F correlate to physicochemical properties (e.g., particle agglomeration state, nominal tube diameter, etc.). Alveolar fibrosis was measured using morphometric point and intercept counting, and was generally increased in 7 of the 9 materials reaching significance in materials with nominal tube diameter greater than or equal to 50 nm. Tracheo-bronchial lymph node (TBLN) and liver sections at 84 d post-exposure were microscopically evaluated for particle accumulation. A range of TBLN and lung accumulation patterns were observed, which reflect the ability of macrophages to phagocytose and clear particles dependent on size and agglomeration. Systemic translocation was limited to single tubes or fibers rather than agglomerates, meaning less systemic accumulation for smaller diameter, more agglomerated material. The lower dose of MWCNT did not exceed a lung to blood distribution, even though nanofibrous material was measured using morphometric point and intercept counting, and was generally increased in 7 of the 9 materials reaching significance in materials with nominal tube diameter greater than or equal to 50 nm. Tracheo-bronchial lymph node (TBLN) and liver sections at 84 d post-exposure were microscopically evaluated for particle accumulation. A range of TBLN and lung accumulation patterns were observed, which reflect the ability of macrophages to phagocytose and clear particles dependent on size and agglomeration. Systemic translocation was limited to single tubes or fibers rather than agglomerates, meaning less systemic accumulation for smaller diameter, more agglomerated material. In conclusion, histopathologic changes and translocation were dependent upon physicochemical properties such as particle agglomeration size and morphology. Ongoing research and modeling techniques will elucidate relationships between physicochemical characteristics and toxicities of various CNT/F.

2219 Multiwalled Carbon Nanotubes Modulate Immune Responses and Pulmonary Injury without Increasing Influenza A Virus Titers in Infected Mice

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Extensive development and use of nanomaterials (NMs) have garnered concerns regarding their potential exposure and adverse health effects. Many toxicological investigations have particularly focused on pulmonary injury that include fibrosis, asthma, and possibly lung cancer, but only a few reports have explored how exposure to NMs can affect host’s susceptibility to pathogenic infections. Previously, we have found exposure of mice to pristine single-walled carbon nanotubes (SWCNTs) can significantly increase viral titers to influenza A virus (IAV) in mice in concert with repressed antiviral and pro inflammatory immune responses. In the present study, we investigated if pre-exposure to a different type of carbon nanotube, hydroxylated multi-walled carbon nanotubes (MWCNTs), would impact immune mechanisms and viral titers similarly. Male mice were randomly assigned to control, MWCNTs, IAV, and MWCNTs+IAV groups and exposed to either 20 µg of MWCNTs or control vehicle (pluronic) by pharyngeal aspiration. On day 3, animals were then given 3.14×10⁵ TCID₅⁰ IAV or PBS by intranasal instillation. All animals were euthanized on day 7 and endpoints including immune cell profiles and cytokine and chemokine levels in bronchioalveolar fluid (BALF), lung histopathology and mRNA expression of innate immune and oxidative stress-related genes, were measured. Our results showed minimal lung tissue damage when mice were exposed to MWCNTs alone. Compared with mice that were exposed to IAV only, MWCNTs didn’t significantly alter viral titers or immune cell profiles in BALF in the co-exposed group, but significantly increased cytokine and chemokine levels (IL-1β, TNFα, GM-CSF, KC, MIP-1α, MIP-1β, MIP-2, and RANTES), and inhibited expression of antiviral genes (Ifn-γ, Rgp-1, Mda5, and Ifn-2). Unlike pristine SWCNTs, hydroxyl functionalized MWCNTs do not increase viral titers to IAV infection, however they do cause molecular changes related to the immune response that are similar between CNT types.
Silver nanoparticles (AgNPs) are, largely due to their antimicrobial properties, one of the most commonly used nanomaterials in consumer products such as cosmetics, clothing, household products and even in food products and biomedical purposes, including drug delivery and tumor targeting applications. Although reports of human exposure via several routes have been rapidly increasing owing to increases in the manufacture and utilization of AgNPs, few reports exist on the acute toxicity associated with different sizes of AgNPs. Recently, we reported that intraperitoneally administered AgNPs in 10 nm diameter showed acute toxicity in BALB/c mice, e.g., reduced activity and piloterection at 5 hours post-administration, lowered body temperatures at 6 hours post-administration and death within 24 hours post-administration with histopathological lesions mainly in liver, but the same amount of AgNPs in 60 and 100 nm diameter did not. This study aimed to evaluate the effects of antioxidants on acute toxicity of intraperitoneally administrated AgNPs in BALB/c mice. One hour after the administration of AgN-acetyl-L-cysteine (NAC, 2000 mg/kg bw, i.g.), Vitamin C (200 mg/kg bw, i.g.), Vitamin E (100 mg/kg bw, i.g.) or L-buthionine-(S,R)-sulfoximine (1.6 g/kg bw, i.p.), 10 nm AgNPs (0.2 mg/mouse) was intraperitoneally administered to 7-week-old female BALB/c mice (5 mice/group) and then sacrificed 6 and 24 hours after AgNPs treatment. In biochemistry, glucose was increased in all AgNP treated groups, except for NAC + AgNPs group. In histopathology, the lesions observed in AgNPs only group, congestion, vacuolation in the liver, increased cellular components in the liver sinusoid and apoptosis in white pulp of the spleen were found at 6 hours, while these lesions were eliminated by NAC pre-treatment but not by others. These lesions were remained until 24 hours. Acute toxicity of intraperitoneally administered 10 nm AgNPs was reduced by only NAC of pre-treated antioxidant.

Cytogenotoxic and Mitodepressive Effects Induced by Silver and Copper Oxide Nanoparticles, and Their Mixture in Allium cepa L.  
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Silver (Ag) and copper oxide (CuO) nanoparticles (NPs) are utilised in the manufacture of numerous consumer products because of their antimicrobial properties. However, there are scientific concerns that their potential release and coexistence in the environment may lead to genotoxic consequences in the ecosystem. Therefore, sufficient information on the cytogenotoxicity of both NPs and none on their mixture. Therefore, we investigated the cytotoxic and genotoxic effects of AgNPs, CuONPs and their mixture (1:1) using the Allium cepa chromosome aberration assay. The nanoparticles were characterised using Transmission Electron Microscopy and Dynamic Light Scattering. Allium cepa roots were exposed to five concentrations (5 - 80 mg/l) of each NP and their mixture for 24 h; and the recovery effect was examined after another cell cycle in distilled water. Both NPs were spherical in shape. AgNPs, CuONPs and their mixture respectively had hydrodynamic sizes of 219.40 nm, 2260 nm, and 282.50 nm; and zeta potentials of -25.30 mV, 5.29 mV and -16.40 mV. AgNPs significantly (P < 0.05) increased Mitotic Index (MI) while CuONPs and their mixture significantly (P < 0.05) decreased the MI. Chromosomal aberrations such as sticky chromosomes, disturbed spindles, anaphase bridges were significantly (P < 0.05) increased in A. cepa exposed to both NPs and mixture compared with the negative control. Partial recovery effect (36 %) was observed in the root cells exposed to AgNPs only. Interaction factor analysis of the mixture showed that the AgNPs and CuONPs interacted antagonistically to induce cytogenotoxicity. These results show that exposure to AgNPs, CuONPs and their mixture are capable of inducing chromosomal anomalies in Allium cepa; and may be of environmental and public health importance.

Changes in Lung and Blood Transcriptomes Following Exposure to Multi-walled Carbon Nanotubes in Mice  
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Pulmonary exposure to multi-walled carbon nanotubes (MWCNT) has been shown to cause local acute and chronic inflammation, fibroproliferation, and immunotoxicity in small animal studies. However, the search for representative biomarkers of exposure is an ongoing endeavor. Whole blood gene expression profiling is a promising approach to identification of novel biomarkers for tissues over traditional histopathology. This study was to look for correlates between RNA expression in lung tissue and in whole blood after MWCNT exposure. Mice were administrated 40 µg of MWCNT or USP-grade phosphate buffered saline via pharyngeal aspiration and sacrificed 56 days after treatment. RNA microarray (Agilent 2100) studies were performed alongside histopathology of lung sections. Analysis of lung tissue RNA using Ingenuity Pathway Analysis (IPA) indicated trends in inflammatory response and connective tissue organization markers, with an emphasis on immune cell trafficking, phagocytosis, and adaptive immune responses. At the same time, many innate immunity-related transcripts (plunc, bpi1, reg3g) were significantly downregulated. Activation of cancer-related disorders and functions was mostly decreased. IPA analysis of the 280 significantly differentially expressed genes in the whole blood was suggestive of increased hematopoiesis and cell development, activation of cancer/tumor development pathways, and atopy. Additionally, gene expression changes in the blood were indicative of ER-stress and mTOR pathway. The goal of this study was to identify common, upregulated genes between whole blood and lungs, important for the adaptive immune responses: ccx1r, cd72, sharpin, and s1cl1a1. Trim24, important for T,2 cell effector function, was downregulated in both datasets. Hla-dqa1 (MHC-I1) mRNA was upregulated in the lungs and downregulated in the blood, as was ilr4b, which controls intracellular response. The only common disorder between lungs and blood was “hypersensitivity.” In conclusion, our studies have shown that transcription changes occurring in the lungs of MWCNT-exposed mice do not necessarily produce a completely replicable pattern in the whole blood, however, specific systemic responses may be shared between transcriptomic profiles.

Development of Whole Body Inhalation System for Well-Dispersed Nanomaterials Toxicity Testing (Taquann Direct-Injection Whole Body Inhalation System)  
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Inhalation is a primary route of exposure to nanomaterials (NMs) and therefore the whole-body inhalation exposure studies are most important for their toxicity testing. In designing inhalation toxicity studies for NMs, it is important to control the status of aggregate and agglomerates (AA) to be consistent with the status presumed in human exposure. For example, a multiwalled carbon nanotube (MWCNT) such as Mitsui MWNT-7 is a mixture of single fibers (SF) and their AA. Human ambient air, in general, is less agitated; the AA may sediment away faster, single fibers may suspend longer in the air and be inhaled by humans. On the other hand, the air for experimental animals is rigorously agitated in most studies, which could control the reactivity of the aerosol: when the aerosol is a mixture of SF and AA, the AA could be trapped by the upper respiratory tract and impede the SF to reach the alveolar region and consequently induce AA-specific proximal lesions. Taking all into account, we considered that it is essential to make a well-dispersed aerosol without AA for the human-relevant exposure scenario. To realize this condition, we developed a simple and reproducible dispersion method (Taquann Method) and an optimized aerosol generator system for this dispersed samples, designated as Taquann Direct-Injection Whole Body Inhalation System (Taquann Method). Using this system, we conducted a study to demonstrate its performance. C57BL/6 mice were exposed to pristine MWNT-7 (U-CNT) or Taquann-dispersed MWNT-7 (T-CNT) aerosol at a concentration of 2 mg/m3 for 2 hours per day for 5 days. Lung burden of mice exposed to U-CNT was half of those exposed to T-CNT (approx. 4 and 8 micro-gm per animal, respectively). AA were found trapped by the nasal cavity mucosa in U-CNT group, explaining its lower lung burden. Our preliminary short-term inhalation toxicology tests confirmed that this Taquann method and Taquann inhalation system is applicable to various types of NMs with minimal sample-dependent pre-treatment/pre-adjustment. The latest version is less expensive, and fully
2224 Pulmonary Toxicity Associated with Different Zinc Nanoparticles after Intratracheal Instillation in Rats

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Zinc nanoparticles (Zn NPs) are used extensively in various products including cosmetics, personal hygiene products, paints, food additives, and cancer therapeutic agents. As Zn NPs production increases, pulmonary toxicity related to occupational exposure becomes a concern. The goal of this study was to assess pulmonary toxicity of Zn NPs of different size, shape, solubility, and composition. In the first study, 2 doses (0.05 and 0.125 mg) of 6 particles were examined: fine-sized ZnO (FZnO), nano-sized elemental Zn (EZn), nano-sized ZnO (NZnO), ZnO nanowire (WNZnO), and comparable nano-sized TiO2 (NTiO2) and TiO2 nanowire (WTiO2) as highly insoluble control materials. Male Sprague-Dawley rats were exposed by intratracheal instillation (II) to 0.05 or 0.125 mg of particles or vehicle control DM (dispersion medium) following a 5 min sonication on day 0. Body weights were recorded throughout the study. Parameters of lung injury and inflammation were measured in bronchoalveolar lavage (BAL) at 1, 7, 14, 21, and 28 day post-exposure. In a second study, 5 doses of TIO2 FZnO and EZn were exposed orally (PO) at 0.05 to 0.0 or 0.125 mg of particles or vehicle control DM (dispersion medium) following a 30 sec sonication that resulted in less release of soluble Zn prior to II. The same time points and endpoints as in the first study were analyzed. In the first study, NZnO had the greatest specific surface area (SSA) and solubility prior to II. FZnO and NZnO agglomerates were smaller than the other samples, and consistent across studies. Study 1 showed the high dose of FZnO and NZnO caused decreased body weight on 1-3 D, which gradually increased by 1 M, followed by normal weight gain up to 3 M. All particles caused transient lung injury and inflammation at 1 D post-exposure except NTiO2, with NZnO>FZnO>EZn>WNZnO>WTiO2. At 7 D, lung injury and inflammation remained increased in the groups exposed to Zn NPs only. Inflammation persisted up to 3 M post-exposure to the greatest degree in the NZnO group followed by FZnO then EZn, while there was recovery in the groups exposed to TiO2 and WTiO2. Eosinophils were increased to the greatest degree in the NZnO>FZnO>EZn groups at 1 and 7 D. Study 2 showed a similar trend as the first study in changes of body weight, lung injury, and inflammation, although the difference between the NZnO and FZnO was not as pronounced. Material characterization studies suggested differences in toxicity may be associated with increased solubility as a function of composition (oxide vs elemental) and increased SSA.

2225 Bioactivity of Boron Nitride Nanotube Preparations That Differ in Purity In Vitro and In Vivo

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Boron nitride nanotubes (BNNTs), due to their wide band gap and thermal and chemical stability, are expected to be incorporated into a myriad of industrial applications. Currently, commercial production of BNNTs occurs through different processes including a pressurized vapor/high temperature process (PVTH) or an induction thermal plasma process (plasma), both resulting in 30-60 % residual compounds and impurities. In the current work, we evaluated the pulmonary and systemic toxicity arising due to acute exposure of BNNTs from the plasma process. Four BNNTs with a gradient of purity (from 50% to 90% tubes) were used to assess toxicity and evaluate bioactivity. Hexagonal boron nitride (less than 100 nm in diameter) was used as a reference material. All BNNTs tested were agglomerated bundles of few multi-walled tubes (~3 to 5 walls/tube). Electron microscopy (EM) confirmed a visible decrease in impurities and an increase in tubular structures across the gradient samples. In vitro and in vivo experiments were performed following sonication of BNNTs in dispersion media (DM). Preliminary EM sizing showed that the BNNTs dispersed in DM had a length of ~0.5 - 1.5 µm and a diameter of ~5 - 30 nm. Electrocardiographic and magnetic resonance measurements showed no change in surface hydroxyl radicals among the BNNTs with various purities. In vitro, the toxicity was evaluated in human monocye cells (THP-1) wild-type and NLRP3-deficient cells at a concentration range of 0-100 µg/mL. At the high doses, there was a small but statistically significant increase in lactate dehydrogenase (LDH) released in the highest purity BNNT exposures. This increase in toxicity was attenuated in NLRP3-deficient cells. In vivo toxicity was evaluated in male C57BL/6 mice exposed by oropharyngeal aspiration to 4 or 40 µg of BNNT sample/mouse. Animals were euthanized 1 and 7 d post-exposure and lung lavage was performed to evaluate lung injury and inflammation. At day 1 there was a significant influx in neutrophils, a marker for lung inflammation, as well as an increase in LDH activity in particle-exposed groups. The response was highest in animals exposed to the high dose of the highest purity BNNT mixture. Inflammation and injury began to resolve by 7 d. These results indicate that BNNTs made by plasma processes induce acute toxicity and inflammation only at high concentrations and ongoing studies will evaluate histopathological changes and clearance up to 3 mo post-exposure.

2226 Activities of Mitochondrial Enzymes in Heart and Brain of Rats Exposed to Titanium Dioxide Nanoparticles

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Titanium dioxide Nanoparticles (TiO2 NPs) have found wide application in various products making exposure to this metal oxide nanoparticle inevitable. Information about toxic effects of TiO2 NPs after oral exposure are extremely limited. Since mitochondrionopathies are being recognized as subtle and insidious biochemical events in the toxicity associated with various toxicants, this study determined the activities of mitochondrial enzymes (Malate dehydrogenase, MDH; Succinate dehydrogenase, SDH; Complexes Complete III+III, CIII+III, Complexo IV-IV, CVIP) in rats exposed to TiO2 FZnO and EZn NPs at oral doses (50, 150 and 250mg/kg body weight) for 4, 8 and 12 weeks. Control rats (n=18) received distilled water for the same period. At the end of TiO2 NPs exposure, brain and heart were removed from the rats and activities of mitochondrial enzymes determined. Enzymes in the two organs exhibited different patterns on exposure to TiO2 NPs. While cardiac MDH was down-regulated throughout the study, brain MDH increased at 4 and 8 weeks of 50 and 12 weeks of 150mg/kg body weight doses respectively. In both organs, SDH activity was up-regulated at 4 weeks. While the up-regulation in cardiac SDH was dose-dependent, brain SDH did not show any dose dependency. In contrast however, CPII+III in both organs was down-regulated, except at 4weeks of 150- and 12weeks of 50mg/kg body weight where increases ranging between 2 and 7 folds were observed. While cardiac CIII+ was decreased at 8 weeks of 150mg/kg body weight dose, the brain enzyme was up-regulated by 50 and 250mg/kg body weight doses of TiO2 NPs. Unsystematic changes characterized the response in brain CVIP, whereas the cardiac enzyme was down-regulated, except at 8weeks of 50 and 250mg/kg body weight doses of TiO2 NPs. Our findings indicate that TiO2 NP-induced alterations in cardiac and brain mitochondrial energy metabolism might be important in pathologies associated with exposure to TiO2 NPs.

2227 Crystalline Nanocellulose-Induced Lung Toxicity and Global Gene Expression Changes in the Rat


Crystalline nanocellulose (CNC) is an emerging nanomaterial with multiple commercial and industrial applications. Occupational exposure to CNC during the production and/or use of products containing the nanomaterial potentially resulting in adverse health effects among workers is possible. Therefore, there is an immediate need to determine the toxicity potential and mechanisms underlying CNC toxicity. Male Fischer rats were exposed by whole body inhalation exposure to air or CNC (20 mg/m³, 6 hours/day, 14 days), and pulmonary toxicity and lung gene expression changes were determined one day following the last exposure. CNC particles were detected in the lung alveoli of the exposed rats. Compared with the air-only exposed controls, significant increases (p<0.05) in the incidence of bi-nucleated alveolar macrophages (AM), lactate dehydrogenase activity, pro-inflammatory cytokine levels, phagocyte oxidant production, and AM and neutrophil counts were detected in the bronchoalveolar lavage of the CNC exposed rats. Mild lung histological changes, such as the accumulation of AM and neutrophils, were observed in the CNC exposed rats. Global gene expression profiling by next generation sequencing identified 573 genes whose expressions were significantly different (FDR p<0.05) in the lungs of the CNC exposed rats, compared with the controls. Bioinformatic analysis of the lung gene expression data identified significant enrichment of several biological functions and canonical pathways related to inflammation (cellular movement, immune cell trafficking, inflammatory diseases and response, respiratory disease, and free radical scavenging, complement system, acute phase response, leukocyte extravasation signaling, granulocyte and agranulocyte adhesion and diapedesis, IL-10 signaling, phagosome formation and matu-
2228 The Impact of Neuroinflammatory Disease and Environmental Exposures on Gold Nanoparticle Accumulation in the Mouse Brain


Epidemiological studies correlate air pollution exposure with increased incidence of Alzheimer’s Disease (AD). We postulate that translocation of inhaled ultrafine particles (UFP, <100 nm) to secondary target organs, including the brain, is one mechanism by which airborne UFP can cause adverse effects. The biodistribution of nanoparticles, particularly to the brain, is still a question that needs to be thoroughly investigated. In young, healthy C57 mice (n=7) that were exposed for 4hrs via whole body inhalation (WBI) to gold nanoparticle aerosols (AuNP; count median diameter (CMD), 20nm, geometric standard deviation (GSD), 1.5), AuNP did not readily accumulate in the brain following an inhalation exposure. We hypothesize that inflammatory insults, such as environmental exposures or neuroinflammatory diseases, can change barrier permeability and ultimately alter NP biodistribution. Aged 3xTgAD mice - which mimic human AD-related pathologies and exhibit progressive neuroinflammation - and non-transgenic (NTg) mice (16 mo males, n=7-8) were exposed via WBI to AuNP aerosol for 4 hrs (CMD, 27 nm, GSD, 1.5). Tissues were harvested 24 hrs post-exposure and analyzed for Au using inductive-coupled plasma mass spectrometry. There was no significant difference of Au accumulation by genotype in the brain regions measured. Separate groups of aged 3xTgAD and NTg mice were also exposed to ambient UFP-enriched aerosols for 4hrs/day for 8 days (CMD, 88 nm, GSD, 1.5) as a purported air-blood barrier insult prior to AuNP aerosol exposure to characterize exposure-related changes in AuNP accumulation in the brain. UFP-enriched aerosol exposures did not induce acute lung inflammation nor significantly affect AuNP accumulation in the brain regions measured. However, when 3xTgAD and NTg mice (3, 19, and 23 mo males, n=7-11) were injected intravenously with colloidal AuNP (primary particle size 20 nm, 45-49 μg per mouse), there was a significant age x genotype interaction effect (p=0.006) found in the whole brain, as well as in areas of the brain that are particularly affected by AD, such as the hippocampus (p<0.05). This suggests that as the pathology progresses, the brain becomes more permeable to vascular accumulation of AuNPs, but that stronger insults are needed to see any differences in brain accumulation of inhaled AuNP. Funding: R01ES020332, T32ES007026, 3P30ES001247.

2229 Limited Neurotoxicity from Neonatal Inhalation Exposure to Ultrafine Carbon Particles or Diesel Particulate in C57Bl/6


Epidemiological studies have shown exposure to anthropogenic fine particulate matter is associated with adverse neurodevelopmental outcomes in children. Complementary studies using rodent models have shown that developmental exposure to ambient nanoscale particulate matter can lead to sex-specific neurotoxicity and learning deficits. However it is still unclear about the direct sources and particulate matter constituents that contribute to these deleterious outcomes. To better evaluate potential particulate matter contributors to developmental neurotoxicity, two studies were conducted to assess the potential developmental effects of pure ultrafine carbon particles (UFCP; median aerodynamic diameter: <40 nm) and nanoscale diesel particles (median aerodynamic diameter: <100 nm) on brain pathology and behavior in C57Bl/6 mice. The UFCP aerosol was generated from a spark-discharge setup and the diesel particles were generated from ultrasonic nebulization of dissolved National Institute of Standards and Technology Standard Reference Material 1650b (SRM 1650b). Separate inhalation exposure of each material with neonatal mice occurred on postnatal days 4-7 and 10-13 for 4hrs/day, 4 days/week at a mass concentration of 50 μg/m3 for UFCP and 100 μg/m3 for SRM 1650b. Assessments of central nervous system pathology 24 hours following exposure showed no gross inflammation or injury following pure ultrafine carbon exposure, while SRM 1650b did increase glial fibrillary acidic protein (GFAP) immunodensity in the corpus callosum and cortex, suggestive of inflammation. To assess learning deficits, behavior on a fixed-interval schedule of reinforcement, a paradigm that involves temporal learning and is historically effective at detecting the protracted effects of low-dose neurotoxicants such as lead, was utilized in exposed adult. No significant treatment-related learning differences were found in the adult mice. The lack of extended effect from the developmental particulate matter exposures, even at relatively high mass concentrations, suggests neither ultrafine elemental carbon nor diesel particle exposure alone are sufficient contributors to adverse developmental neurotoxicity. Further research on more reactive constituents of particulate matter including volatile organic species, reactive metals, and gases need to be done to better clarify specific toxic contributors.

2230 Activities of Glycolytic Enzymes in Rats Exposed to Titanium Dioxide (TiO2) Nanoparticles (NPs)


Titanium dioxide nanoparticles (TiO2, NPs) have been applied widely in various products such as food, packaging, electronics, sunscreens, paints, drugs and cosmetics. Safety of TiO2 NPs remains unclear because data on absorption, distribution, elimination or any adverse effects after oral exposure are extremely limited. Since disorders of energy metabolism are being recognized as incipient biochemical events in the toxicity associated with various toxicants, this study determined the activities of glycolytic enzymes in rats exposed to TiO2, NPs. Male albino rats were exposed orally to TiO2, NPs (8-12nm) (50, 150 and 250mg/kg body weight) for 4, 8 and 12 weeks. Control rats (n=18) received distilled water for the same period. Tissues were received at the end of TiO2, NPs exposure, blood and liver were removed from the rats. Activities of glycolytic enzymes (Hexokinase [HK], Aldolase [ALD] and Lactate dehydrogenase [LDH]) were then determined. In the lymphocytes (with the exception of 50mg/kg body weight, 8 weeks and 150mg/kg body weight, 12 weeks), exposure to TiO2, NPs up-regulated HK activity. Plasma LDH activity was also up-regulated except at 12 weeks of 150mg/kg body weight dose where 40 and 30% decreases were observed in the plasma and erythrocytes respectively. Hexokinase followed the same pattern as that of the lymphocytes. In a similar vein, both hepatic and lymphocyte ALD followed the same pattern and exocytosed to TiO2, NPs. Their activities was assessed in the brain regions except at 8 and 12 weeks of 50 and 150mg/kg body weight where slight decreases were observed. While plasma ALD also increased (except at 12 weeks of 150mg/kg body weight), erythrocyte ALD increased only at 4 and 8 weeks, but decreased throughout the 12 week exposure period. Compared to erythrocyte LDH that decreased on exposure to TiO2, NPs, a common signature was observed for the lymphocyte, plasma and hepatic enzymes. LDH in these compartments was down-regulated at 8 and 12 weeks of 50 and 150mg/kg body weight doses of TiO2, NPs, whereas at other doses and time intervals, LDH was up-regulated. Our findings indicate that while exposure to TiO2, NPs inhibited erythrocyte glycolytic pathway at the level of ALD and LDH, it enhanced the flux through the plasma, lymphocyte and hepatic glycolytic pathways.

2231 Effects of Multi-Walled Carbon Nanotube Exposure on Brain Oxidative Stress and Inflammation in C57BL/6 Mice

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In vivo models demonstrate increased brain and lung inflammatory activation, disrupted blood-brain barrier (BBB) integrity, increased oxidative stress, serum profile changes, impaired endothelial function, and vasodilatory insufficiencies following multi-walled carbon nanotube (MWCNT) exposure. The mechanism by which oxidative stress and inflammation are explored in this study. We hypothesized that the MWCNT exposure induces serum peptide composition modifications that mediate pro-inflammatory and pro-oxidative stress changes in the brain, likely delivered via exosomes. To test this hypothesis, male wild-type C57BL/6 mice (6-8 weeks) exposed to vehicle (dispersion media), 3, 10 or 40 μg MWCNT via oropharyngeal aspiration were euthanized at various time points post exposure. Pulmonary inflammation was assessed via bronchoalveolar lavage fluid (BALF) cell and protein quantification and inflammatory cytokine/chemokine expression was determined by electrochemiluminescence. Serum bioactivity of whole plasma MWCNT was significantly decreased in MWCNT-exposed mice. While plasma ALD also increased (except at 12 weeks of 150mg/kg body weight), erythrocyte ALD increased only at 4 and 8 weeks, but decreased throughout the 12 week exposure period. Compared to erythrocyte LDH that decreased on exposure to TiO2, NPs, a common signature was observed for the lymphocyte, plasma and hepatic enzymes. LDH in these compartments was down-regulated at 8 and 12 weeks of 50 and 150mg/kg body weight doses of TiO2, NPs, whereas at other doses and time intervals, LDH was up-regulated. Our findings indicate that while exposure to TiO2, NPs inhibited erythrocyte glycolytic pathway at the level of ALD and LDH, it enhanced the flux through the plasma, lymphocyte and hepatic glycolytic pathways.
staining revealed increased albumin staining in extravascular spaces of exposed mice compared to controls. Studies to evaluate oxidative stress, neuroinflammation, and exosomal contributions are ongoing. These preliminary findings suggest that MWCNT exposure induced a pro-inflammatory milieu that may disrupt the BBB.

**2232 ICONS - Integrated Testing Strategy for Mechanistically Assessing the Respiratory Toxicity of Functionalized MWCNT**

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Multiwalled carbon nanotubes (MWNT) show promising technical properties (e.g., composite reinforcement; electrical conductivity). MWNT, meeting WHO criteria, exhibit straight or curved/tangled morphology, depending on length and diameter. Evident fiber toxicity was observed for the rigid fiber type, i.e. carcinogenicity in the rat model after intratracheal injection. Tangled type MWNT (not carcinogenic) may show an altered toxic potential regarding fibrogenic or genotoxic effects following surface modification with functional groups such as -NH2 or -COOH. The ERA-NET SIINN project ICONS (International Collaboration On Nanotube Safety) aimed at mechanistically evaluating and ranking the pro-fibrotic and genotoxic potential of tangled MWNT, focusing on impact of core purification and surface functionalization. A batch of industrially relevant Nanonyl NC7000 was therefore chemically or thermally purified and surface functionalized (-COOH and -NH2). At Fraunhofer ITEM, the eight resulting MWNT (pristine, milled, purified, and functionalized) were tested for sterility and endotoxin. For in vitro use, they were dispersed using an ultrasound-based protocol, and characterized by light and scanning electron microscopy. Subsequent in vitro (gene)toxicity testing with MRC-5 primary human lung fibroblasts revealed differential induction of proliferation (RICK, mitotic index), induction of membrane damage and micronuclei, and loss of chromosomes. Based on these results and existing in vivo data (generated by LTAP and NCSU; oro-pharyngeal aspiration tests), two functionalized MWNT will be selected for a 4-wk inhalation test in rats (based on OECD TG 412), including a 4-wk recovery (validation test). Completed pre-trials demonstrated feasibility of generating respirable MWNT aerosols by dry dispersion with pressurized air. This project is funded by the German BMBF (FKZ: 03XP0063).

**2233 Analyzing the Mechanism of Amorphous Silica Nanoparticle-Induced Immunosuppression in a Mouse Model of Allergic Contact Dermatitis**


Over the last few decades, the field of nanotechnology has grown swiftly, and nanoparticles (NP) based consumer goods are now widely available. Specifically, amorphous silica NPs are approved anti-caking agents in both foods and cosmetics. Furthermore, additional research suggests a potential role for silica NPs in drug delivery and bio-sensing applications. The ubiquitous nature of NP enabled technology increases the need for comprehensive toxicological evaluation of NPs. Our lab is interested in the effects of topical exposure of silica NPs on both healthy skin and skin models of allergic contact dermatitis (ACD). For this study, hairless C57BL/6 mice were either challenged with vehicle or 2, 4-dinitrofluorobenzene (DNFB), after DNFB sensitization. Topical exposure of only 4 μg/ear of 20 nm silica NPs decreases the DNFB induced ear swelling response, and this effect is associated with decreased inflammatory cytokine expression (IL-1β and MIP-2) and reduced cytotoxic T cell skin infiltration, after 24 hours. Importantly, we have also identified that silica microparticles (400 nm) do not reduce the DNFB induced ear swelling response, even with equivalent surface area doses. This suggests that reductions in DNFB bioavailability in the skin, due to particle binding, is an unlikely mechanism of action. To evaluate the upstream signaling events in the silica NP treated skin, we performed a global RNA sequencing study to assess NP induced transcriptome changes in whole skin samples. Over 3400 genes were differentially expressed in DNFB treated skin, compared to control; however, the expression of only 28 genes were significantly altered by 20 nm silica NP exposure in DNFB treated skin. Genes associated with lipid synthesis, extracellular matrix based signaling, activator protein-1 signaling, and anti-oxidant activity were all upregulated by silica NP exposure. While additional studies are required to identify whether these changes in the transcriptome lead to functional changes, these genes represent a potential mechanism by which silica NPs reduce skin inflammation. Supported by: NIH Training Grant ES-05026, R01 ES014299.

**2234 Nickel Nanoparticles-Induced Lung Injury and Fibrosis: The Role of miR-21**

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In previous studies, we and other groups have demonstrated that exposure to nickel nanoparticles (Nano-Ni) causes severe and persistent lung inflammation and fibrosis. Although it is well-known that Nano-Ni induces oxidative stress, it is unclear which mechanisms contribute to lung fibrosis. Here, we propose that miR-21 may play an important role in Nano-Ni-induced lung inflammation, injury, and fibrosis. miR-21 knock-out (miR-21 KO) and their wild-type (WT) (C57BL/6) mice were used for our in vivo comparative studies. Mice were intratracheally instilled with 50 μg per mouse of Nano-Ni. At day 3 post-exposure, bronchoalveolar lavage (BAL) was performed and the neutrophil count, CXCL1/KC level, LDH activity, total protein concentration, and MMP-2/9 protein levels and activities in the BALF were determined. In addition, mouse lung tissues were collected for determination of MMP-2/9 activities by gelatin zymography assay and for histology study by H&E staining. At 6 weeks post-exposure, mouse right lungs were collected for determination of hydroxyproline content. At day 3 post-exposure, 50 μg per mouse of Nano-Ni caused severe acute lung inflammation and injury that were reflected by increased neutrophil count, CXCL1/KC level, LDH activity, total protein concentration, and MMP-2/9 protein levels and activities in the BALF from WT mice. Nano-Ni exposure also caused increased MMP-2/9 activities in the mouse lung tissues from WT mice. Although Nano-Ni also caused above effects in miR-21 KO mice, their levels were significantly lower than those in the WT mice. Histologically, infiltration of a large number of neutrophils and macrophages in the alveolar space and interstitial tissues was observed in lungs from WT mice exposed to Nano-Ni. However, exposure of miR-21 KO mice to Nano-Ni only caused mild acute lung inflammation and injury. At 6 weeks post-exposure, in the WT mice, Nano-Ni caused extensive interstitial fibrosis and proliferation of interstitial cells with inflammatory cells infiltrating the alveolar septa and alveolar space. However, exposure of miR-21 KO mice to Nano-Ni only caused slight lung fibrosis. Our results demonstrate that short-term Nano-Ni exposure causes less acute lung inflammation and injury in miR-21 KO than WT mice. In addition, long-term Nano-Ni exposure in miR-21 KO mice causes much milder chronic lung inflammation and fibrosis than that in WT mice. Our results suggest that miR-21 may be involved in Nano-Ni-induced lung injury and fibrosis.

**2235 Pharmacokinetics Relates to Safety of a Gadolinium-Containing Metallofullerene**

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The pharmacokinetic studies used male rats given IV doses of 2 mg/kg of a gadolinium-containing metallofullerene proposed for use in diagnostic imaging. Gadolinium content in a 2 mg/kg dose of the dosing solution was 0.53 mg/kg, as determined by ICP-MS, using a method with a detection limit <0.05 ng/ml in solution. Gadolinium concentrations in whole blood samples taken 15 to 24 h after Gd-metallofullerene administration showed rapid depletion of the quantity of the test agent (half life < 2 h), with levels at 16 h less than 5% of what they were at 1 h. Gd contents in liver samples measured 16 h after dosing (n=3) were 217, 145 and 233 ng/g of liver. These levels were higher than levels in blood at that time and approximated those levels measured in blood at 0.5 hr. The test agent also underwent comprehensive safety evaluation after intravenous administration to male and to female rats at the same dose used for pharmacokinetic studies (2 mg/kg of the gadolinium-containing metallofullerene) as well as a dose 5-fold higher (10 mg/kg). Evaluation endpoints included multiple physiological, behavioral and neurological observations done in intact animals before and up to 14 days after test agent administration. In addition, blood samples were taken for clinical pathology and multiple tissues collected for detailed histopathology 2, 4, 8 and 14 days after administration. No agent-induced effects of toxicological significance were noted at any endpoint at either dose in either gender of rats. Results suggest that, at the doses tested, this fullerene was safe but would have higher levels in liver than in blood at 16 h after administration. Supported by NIH to Luna Nanoworks with subaward to Virginia Tech.
We recently reported that modeled acute exposure to multi-walled carbon nanotubes (MWCNT) increased systemic inflammation, induced vascular dysfunction, disrupted the blood-brain barrier (BBB) and induced neuroinflammation. We further identified a dramatic shift in circulating peptides, found to be mediators of systemic bioactivity and a promising source of health-effect biomarkers. Here, we assessed the burden of repeated MWCNT exposure in inciting a peptidomic response, its association with MMP-9 proteolysis, and longer term neuroinflammatory ramifications. Male C57BL/6 wild-type (WT) and MMP-9 <sup>-/-</sup> knockout (KO) mice were exposed to MWCNT-7 by oropharyngeal aspiration (n=7/group): 0 µg control vehicle (0.6 mg/ml albumin, 0.01 mg/ml DPPC) once per week, 10 µg once per week, and 40 µg at week-1 followed by 0 µg once per week until collecting serum and brains at 28 days after the initial treatment (7 days after the last aspiration). An enriched-peptide fraction was extracted and assessed by untargeted data-independent mass spectrometry while brains were assessed by immunofluorescence microscopy. In WT mice, 1613 (34%) of 4759 reproducibly quantified serum peptide factors were significantly responsive across all exposures (5% FDR) with half (785) directly dependent on MMP-9, speaking to the sizeable, though non-exclusive, role of MMP-9 in production of the MWCNT-responsive peptidome. Repeated 10 µg exposure significantly induced 957 (59%) of the peptidomic responses, highlighting the significance of repeated lower dose exposures. Separately, 1119 (69%) peptide factors were significantly shifted a full 4-weeks after the larger 40 µg bolus dose, supporting a prolonged impact to circulating factors. Likewise, albumin leakage across the BBB and pronounced microglial activation proximal to impacted vessels were observed after repeated low dose exposures as well as after 4-week recovery from high dose MWCNT. These effects were strikingly muted in brains of MMP-9 KO animals. Overall findings affirm significant MWCNT effects on the circulating peptidome, a sustained neurotoxicological burden of exposure, and a dependence on MMP-9 proteolytic function, together substantiating a prolonged impact of exposure.

### Systematic Comparative Assessment of the Cellular Stress Response Pathway Activation by Various Valproic Acid Analogues to Support Biological Read Across

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Chemical read across is commonly evaluated without considering mechanistic biological knowledge. Here we used a large panel of valproic acid (VPA) analogues to include detailed mode-of-action (MoA) data as a proof-of-concept for read across. VPA is used as an anticonvulsant, but can induce liver steatosis. In rodents, VPA and some of its analogues cause hepatic steatosis, whereas other analogues do not. Since hepatotoxicity is linked to cellular stress pathway activation, we anticipated that VPA analogues that cause steatosis would activate similar stress response pathways, whereas in vivo negative compounds do not. Therefore, we deployed a panel of cellular stress response HepG2 BAC-GFP reporter cell lines, and performed high throughput transcriptomics analysis of cellular stress pathways. We measured stress response pathway activity at high throughput in the reporter cell lines with SRXN1 as target protein for oxidative stress, BiP for unfolded protein response, and p21 for DNA damage response. Cells were treated with a concentration range of 18 different VPA analogues for 24 h, and then imaged by confocal microscopy followed by quantitative image analysis. We were able to largely predict the steatosis in vivo potential of VPA analogues based on the expression of SRXN1-GFP and p21-GFP. Subsequently, we checked whether VPA analogues that cause liver steatosis yield similar transcriptional perturbations. Tempô-Seq analysis of ~3000 genes involved in stress pathway responses for different VPA analogues in a concentration range over 24 h confirmed the concentration response activation of both oxidative stress and p53 downstream targets as well as additional stress pathways, supporting the application of Tempô-Seq technology. Further comparative analysis among all VPA analogues in both HepG2 and primary human hepatocytes will be discussed. Thus, by monitoring adaptive stress response pathways at both the protein and the transcription level, we anticipate to support risk assessment by providing quantitative and mechanistic biological information to corroborate a robust read-across approach. This work was part of the EU-ToxRisk project and received funding of the European Union’s Horizon 2020 research and innovation program under grant agreement No 681002.
Mitotoxicity perturbation has been recognised as a key event in the process of chemical-induced organ toxicity. Nowadays the assessment of the mitochondrial functioning in early toxicity screens is based on static measurements of various mitochondrial processes. Here we focused on the integration of concentration- and time-resolved imaging-based mitochondrial injury data and subsequent transcriptionomics-related cellular perturbations information. We used a panel of ~20 agrochemicals that specifically target mitochondrial respiratory complex (MRC) I, II or III. Effects of the various inhibitors on mitochondrial functioning were monitored using time-resolved high content confocal imaging of mitochondrial membrane potential (MMP) and cell viability in HepG2 cells. A phenomenological computational model was used to describe the observed MMP dynamics. To link the mitochondrial perturbation to subsequent cellular stress responses, we used TempO-Seq targeted RNAseq assessing >3000 genes that capture the genome wide variations in gene expression upon MRC inhibitor exposure. In addition, BAC-GFP cellular stress response reporter imaging was used to validate the observed mitochondrial and cell injury-related changes in gene and related protein expression. The various CI and CIII inhibitors, but not CII inhibitors, were effective in depleting the MMP. The MMP dynamics were quantified by fitting sets of time constants, which could distinguish the effects of MRC inhibitor treatment. Potency of MMP depletion by MRC inhibitors was related to cell death only when glycolysis was inhibited. In repeat dosing regimens of HepG2 3D spheroids demonstrated the intrinsic mitotoxicity-induced cell death of CI and CIII inhibitors. The TempO-Seq data revealed activation of cellular stress response pathways that typically paralleled the potency of onset of MMP depletion by the MRC inhibitors. Transcriptional induction of stress response markers, as identified by TempO-Seq, could be validated using HepG2 GFP-reporters. In summary, the integrated assessment the dynamics of mitochondrial dysfunction and cellular stress response activation provides a novel approach to quantitatively in-depth assess mitotoxicity liability. This work was part of the EU-ToxRisk project and received funding from the EU’s Horizon 2020 programme under grant agreement No 681002.

Acute kidney injury (AKI) is associated with substantial morbidity and mortality and is recognized as a leading cause of chronic kidney disease. The death of epithelial cells in the proximal tubules is thought to be the primary cause of AKI, but epithelial cells that survive kidney injury have a remarkable ability to proliferate. Because proximal tubular epithelial cells play a predominant role in kidney regeneration after damage, a potential approach to treat AKI involves targeting the epithelial cell proliferation following acute damage in vitro. This compound may provide a path for new therapies toward kidney tubule repair after damage.

The adverse effects of Environmental estrogen, bisphenol A (BPA), are multifaceted; this study was to identify the biological pathways perturbed by BPA that potentially influences the immune response system. Tandem-MS-Tag (TMT)-Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) quantitative proteomic analysis of the proteome of BPA-treated caco-2 cells. Label-Free-Quantification (LFQ) of the proteome of BPA (10 µg/mL) infected mouse CD4+ T cells. Co-immunoprecipitation (Co-IP) with STimulator of Interferon Genes (STING) and ZDHHC1. Cellular stress, phagosome maturation and especially the Siruin signaling pathways were identified, using both IPA and STRING analysis, as the major protein pathways perturbed by BPA in both human caco-2 cells and mouse CD4+ cells. IPA-Upstream regulator analysis indicated that the pro-inflammatory cytokines including IL-4 and IL-5 typically enriched in type 2 T helper cell (T_{h2}) and Arylhydrocarbon Receptor (AhR), were activated in BPA-infected mouse CD4+ T cells. CD38/ cyclic ADP ribose hydrolase or cyclase was another BPA-activated upstream regulator indicated by the proteomics data. A protein palmitoyltransferase, ZDHHC1, was identified by mass spectrometry as one of the top proteins upregulated by BPA. Results from co-immunoprecipitation (Co-IP) demonstrated that ZDHHC1 and STING form a heteromultimeric complex. Through proteomics analysis of BPA-treated or infected cells, we identified the major protein pathways that are regulated by BPA. Typically, the up-regulated oxidative-stress pathway as well as the protein expression of CD38 suggested an increased consumption of NAD+ and the reciprocal down-regulation of Sirt1 that increase histone acetylation and gene activation. Upstream regulator analysis indicated BPA-infected CD4+ T cells presented a pro-inflammatory state of type 2 helper cell (T_{h2}). We found that the expression of ZDHHC1 is up-regulated additively by BPA and vitamin D.

AhR is a nuclear receptor that responds to xenobiotics by induction of genes BPA and CYP1A2, Sustained AhR activation has been associated with carcinogenicity and other toxicities in rodents and humans. Thus, identification of this liability is important to ensure patient safety. We assessed the transcriptional response of AhR genes in liver and lung in a 1-month rat study with 5 and 8-day peel-offs with an AhR-activating carcinogen (PCB-126) at doses with and without treatment to carcinogens (1000 ng/kg/day or 500 nkd) and non-carcinogenic (150 nkd) in 2-y rat studies. For liver, Cyp1a1 demonstrated dose and time-dependent increases with the 1000 nkd dose yielding a median response of 8000-fold in males and females. At the non-carcinogenic dose of 150 nkd, the median at 1 month was 2430-fold for females and 1370-fold for males. Cyp1a2 showed dose and time-dependent increases with the greatest median response of 20-fold at 1000 nkd, while 150 nkd had a response of approximately 8-fold. Cyp1b1 had the greatest response at 1000 nkd at 1 month, with a median response of 2060-fold for females and 1420-fold for males, while at 150 nkd the response was 132-fold in females and 20-fold in males. The response for lung was more variable than in liver, with Cyp1a1, AhR and Cyp1b1 demonstrating informative dose and time-dependent increases. This study characterized transcriptional responses for AhR regulated genes in liver and lung for PCB-126 dosed at a non-carcinogenic (150 nkd) and carcinogenic dose (1500 nkd) for 5 days, 8 days and 1-month, demonstrating that transcriptional responses can also clearly separate these doses. The data from this study could be considered a benchmark for assessing dose-dependent AhR activation and subsequent utility for projecting tumorigenic doses for other compounds, when sustained AhR activation is seen in liver and/or lung.
Ingestion of or exposure to chemicals poses a serious health risk. Early detection of cellular changes induced by such events is vital to identify appropriate countermeasures to prevent organ damage. The aim of this study is to predict in vivo organ injuries in rats with the use of gene expression from in vitro primary liver and kidney cells exposed to thioacetamide, a known liver toxin that promotes fibrosis. A set of gene modules, associated with specific organ injuries, has been derived from gene expression levels in liver and kidney tissues from rats exposed to diverse chemical insults. First, the injury-associated gene modules were assessed and validated by analyzing gene expression data in liver, kidney, and heart tissues obtained from Sprague-Dawley rats exposed to thioacetamide. The rats were injected with low (25 mg/kg) or high (100 mg/kg) dose of thioacetamide for 6 or 24 hours. Finally, the modular approach was used on the gene expression data from primary liver cells (in vitro) exposed to thioacetamide to predict organ injuries observed in vivo. It was found that the most activated liver gene modules in vivo were those associated with cellular infiltration and fibrosis. Histological analyses supported these results, signifying the potential of aerosol gene expression data to identify organ injuries. It was also found that the in vitro to in vivo predictions correlated well with an R² value of 0.64. The top ranking liver injuries in vitro correctly identified known pathological changes such as fibrosis.
Metabolite Markers for Acetaminophen-Induced Liver Damage in the Laboratory Rat

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Acetaminophen (APAP) is the most commonly used analgesic and antipyretic drug in the world, yet poses a major risk of liver injury when taken in excess of the therapeutic dose. Current clinical markers do not detect the early onset of liver injury associated with excess APAP, information that is vital to reverse the injury progression through available therapeutic interventions. Hence, novel injury markers that can detect the onset of liver damages are desirable. Several studies have used transcriptomics, proteomics, and metabolomics technologies independently and in combination to discover potential markers of liver injury. However, the causal relationship between these observations and their relation to the APAP mechanism of liver toxicity is not clearly understood. Here, we used Sprague-Dawley rats orally gavaged with a single dose of 2 g/kg of APAP to collect tissue samples from the liver and kidney for transcriptomics and blood and urine samples for metabolomics analysis. We developed and used a multi-tissue, metabolism-based physiological modeling approach to integrate these data, characterizing the effect of excess APAP levels on liver metabolism, and identified a list of plasma and urine metabolites that are associated with APAP-induced liver toxicity. Our analysis indicated that the amino acid related pathways and nucleotide metabolism pathways in the liver were the major pathways impacted within 10 h post-APAP treatment and metabolites in these pathways could serve as potential markers for APAP-induced liver injury. The coupled multi-tissue modeling framework of in vivo metabolism provides both mechanistic insights and a capability to propose plasma and urine metabolites as potential early markers of toxicant-induced organ damage.

A Computational Biology Framework for Modeling Multisystem Adverse Outcome Pathways Initiated by Exposures to Ozone and Associated Air Pollutants


Exposure to ozone and associated photochemical air pollutants constitutes a persisting and widespread problem around the globe. In addition to causing respiratory effects, photochemical pollution impacts the cardiovascular, immune, integumentary and other physiological systems. Reactive contaminants such as ozone typically exert their detrimental effects via the generation of reactive oxygen species (ROS). For example, ROS result from reactions of ozone with skin lipids during dermal contact and from reactions with lung lining fluid components following inhalation. These secondary ROS initiate a series of cascading events, such as release of pro-inflammatory mediators, infiltration of immune cells, and activation of aryl hydrocarbon receptor (AhR) pathways. Multiple physiological systems are affected by these events; for example, respiratory-provoked pro-inflammatory mediators can enter the circulatory system, initiate neuroendocrine-immune crosstalk and subsequently affect heart rate variability. This work describes the development of interconnected computational simulation modules for the exposure biology of ozone and associated photochemical pollutants in the integumentary, respiratory and cardiovascular systems. These modules are designed as components of the MENTOR (Modeling Environment for Total Risk) modeling platform for whole-body human exposure, dosimetry, toxicokinetics and toxicodynamics. MENTOR employs a spectrum of systems dynamics modeling approaches, combining differential equation and agent-based methods to quantify overlapping Adverse Outcome Pathways (AOPs) involving multiple scales (biomolecular, cellular, histological, organ) and physiological systems and endpoints. Using this approach, results have been obtained for various case studies that demonstrate how our model mechanistically simulates events taking place at the molecular and cellular scale to predict and quantify outcomes at the tissue and organ scale; for example, the model reproduces changes in macroscopic lung function properties (elastance and resistance) that are initiated by reactions of ozone with phospholipids and proteins of the pulmonary lining fluid. Supported by NIH grants ES050522 and ES047483.

Systematic Transcriptomics-Based Comparison of Cellular Stress Response Pathways Activation in Different Human Liver Cell Test Systems

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Drug-induced liver injury (DILI) remains a major burden on the economy and healthcare. Given the diversity of DILI types a improved mechanism-based testing strategy to evaluate DILI liability is essential. Previously we generated a large panel of BAC-GFP HepG2 hepatocyte reporter cell lines in which we can visualize and quantify the abundance and subcellular localization of cellular stress biomarkers. These HepG2 reporters can be used in a high content and throughput imaging-based screening approach to assess safety liabilities of new chemical entities. We applied BioSpyder targeted RNAseq to explore the expression profiles of ~3000 toxicity related genes. We evaluated the activation of four key cellular stress response pathways: oxidative stress (induced by exposures with di-ethyl maleate), unfolded protein response (by tunicamycin), DNA damage response (by cisplatin) and inflammation response (by TNF). In this study we investigated the hepatotoxic responses of 18 BAC-GFP reporters and compared these with wildtype HepG2 responses. We found that the GFP tags did not affect the stress response activation patterns. Next, we benchmarked these responses of HepG2 against other in vitro hepatotoxicity models: stem cell derived hepatocytes (hiPSC-Heps), undifferentiated stem cells (hiPSC) and primary human hepatocytes (PHHs) from 3 different donors. Interestingly, we found that although the hiPSC-heps were immature the stress response activation patterns had a high correlation with responses from PHHs. In general, HepG2 and hiPSC-heps seem more sensitive to cellular stress than PHHs, as their stress activation was already observed at lower concentrations. In terms of robustness the HepG2 outperformed hiPSC-heps and PHHs. Variability in PHHs was especially large for expression patterns of inflammation related genes, likely caused by the hepatocyte extraction process from the donor liver which is known to cause high intra-liver variability levels. To our knowledge this is the first study that systematically studied the expression patterns of different in vitro DILI models under conditions of dedicated cellular stress response activation in hepatotoxic conditions. Supported by EU-ToxRisk project (grant agreement No 681002).

Evaluation of Systemic and Microbiome-Derived Effects of Antibiotics on the Plasma Metabolome in Rats

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Xenobiotics can alter the gut microbiome’s composition and functionality and thereby modulate host health. Metabolomics is a promising tool to identify and evaluate the effects of xenobiotics on the gut microbiome. In previous research, indole derivatives, hippuric acid (HA) and bile acids have been identified as key metabolites affected by antibiotic treatment. This study aims to identify the role of the microbiome in these alterations of metabolic profile after oral and parenteral administration of antibiotics. In this project, two poorly bioavailable antibiotics (vancomycin and streptomycin) were either orally or parenterally administered to Wistar rats to distinguish between microbiome-derived and systemic changes in the plasma metabolome. These reference profiles were compared to the profiles of a third readily bioavailable antibiotic (roxithromycin). 250 defined metabolites were analyzed in plasma of orally and parenterally treated animals, as well as diet and vehicle controls after 7, 14 and 28 days. Indole-3-acetic acid (I3A) and HA were significantly downregulated after both parenteral and oral administration of all three antibiotics. Hereby, I3A and HA seem to be sensitive indicators of gut microbiome modulation, since it is assumed that a small amount of the parenterally administered dose of the antibiotic ends up in the intestinal tract via biliary excretion. Contrary, oral administration of all three antibiotics resulted in alterations in the bile acid pool, with taurocholic acid upregulated and glycochenodeoxycholic acid (GCDCA) downregulated. GCDCA was downregulated after intraperitoneal administration of vancomycin, but not after subcutaneous administration of streptomycin. As roxithromycin has a high bioavailability and is excreted in the feces after parenteral administration, it is in line with expectations that after both oral and subcutaneous roxithromycin treatment, taurocholic acid was upregulated and GCDCA was downregulated. These results show that modulation of the gut microbiome by the poorly bioavailable antibiotics leads to specific changes in especially the bile acid pool that cannot be found after parenteral administration of these anti-
Analysis of Metabolome Changes in the Bile Acid Pool in Feces and Plasma of Antibiotic-Treated Rats

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The bile acid-liver-gut microbiota axis plays an important role in the host’s health. A change of the gut microbiota can impact on the bile acid pool, but also the bile acids themselves can influence the gut microbiota composition. In this study, six antibiotics from five different classes (lincosamides, glycopeptides, macrolides, fluoroquinolones, aminoglycosides) were used to modulate microbial communities of Wistar rats to elucidate changes in the bile acid metabolism and to identify key metabolites in the bile acid pool related to gut microbial changes. 20 primary and secondary bile acids were analyzed in both stool and feces of treated animals, vehicle control and diet control. After treatment, significant changes of primary and secondary bile acids in both matrices of treated animals could be observed. There was an increase of taurine-conjugated primary bile acids in both plasma and feces. Contrary, cholic acid and most of the analyzed secondary bile acids were found to be significantly downregulated in plasma whereas cholic acid accumulated in the feces. This accumulation was not seen for ursodeoxycholic acid indicating a different mode of action for the macrolide antibiotic. Although different classes of antibiotics with different activity spectra against gut microbiota were applied, the overall effect on the bile acid pool tended to be similar in both matrices except for streptomycin because it is not able to cross the intestinal barrier. The bile acid microbiome community affect the bile acid pool in plasma and feces of the host and that bile acid profiling can be indicative for an alteration of the gut microbiome. Further, this change in the bile acid pool could lead to secondary effects such as an altered absorption or excretion of metabolites or xenobiotics or activation or inactivation of nuclear receptors in the liver or intestine which might have implications for toxicological evaluations regarding the gut-liver axis, the immune system and other body functions known to be influenced by the gut microbiota.

Development of a Quantitative Systems Toxicology Model of Drug-Induced Cholangiocyte Injury in DILysim

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Cholangiocyte injury accounts for a quarter of drug-induced liver injury (DILI) cases and is associated with higher rates of morbidity and mortality than other types of DILI (Chalasani et al., 2015). While there are many compounds known to cause cholangiocyte injury, there are currently no methods to screen drugs for this potential at the lead candidate selection phase. Inhibition of multi-drug resistance protein 3 (MDR3) has been shown to correlate with cholangiocyte injury and drug toxicity in humans. MDR3 mediates transport of phospholipids to the bile canaliculus. Inhibition of phospholipid transport reduces micelle formation resulting in naked bile acids that can damage cholangiocytes. Objective: The goal of our project was to develop a novel QST model for assessing and predicting drug-induced cholangiocyte injury in humans. Methods: We developed a cholangiocyte life cycle model as well as a model of phospholipid transport via MDR3. The parameterization of the model relied on experiments on the relationship between phospholipids and bile acids measured in liver transplant patients; specifically, results showing that increases of ALP, believed to reflect cholangiocyte injury, follow an increase in the ratio of bile acids to phospholipids following liver transplant. The submodel was then successfully integrated with DILysim, a QST model of drug-induced liver injury that is being developed by a public-private partnership (The DILysim Initiative). Results: In our submodel, with a simulated MDR3 inhibitor having a Ki of 1 µM, the bile acid to phospholipid ratio increased to ~20 from the baseline level of 10. Total cholangiocyte apoptotic flux increased to cause cholangiocyte injury and drug toxicity in humans. MDR3 mediates transport of phospholipids to the bile canaliculus. Inhibition of phospholipid transport reduces micelle formation resulting in naked bile acids that can damage cholangiocytes. This in silico model can be used to predict the effects of a potential therapeutic candi-
2256 Inter-individual Variability of Carcinogenic Metabolism in the Collaborative Cross Mouse Population

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Human studies often reveal large inter-individual variation in response to chemical exposure, believed to be derived from social, racial, and regional differences. The Collaborative Cross (CC) mice were created to represent a genetically heterogeneous population covering all possible genotypes. We hypothesize that the variability in susceptibility derived from genetic diversity can be modeled in mice by combining the CC model with the 1,3-butanediol (BD) exposure model. BD is metabolized by CYP2E1 to three epoxides that form protein adducts. The BD-derived protein adducts are suitable biomarkers for BD uptake, metabolic activation, and detoxification. Mice from 60 CC inbred strains were exposed to BD and N-terminal valine adducts N-(2-hydroxy-3-buten-1-yl)-valine (HB-Val), N,N-(2,3-dihydroxy-1,4-butanediyl)-valine (pyr-Val) and 2,3,4-trihydroxybutyl-valine (THB-Val) were quantitated. CYP2E1 activity in liver microsomes were determined using P-nitrophenol as substrate. The distribution of N-terminal valine adducts were 5, 17, and 87% for HB-Val, pyr-Val and THB-Val, respectively. For individual exposure groups, CYP2E1 activities initially decreased and then increased with exposures. In contrast to the protein adducts, variation in CYP2E1 activities were less dramatic, and ranged from 5 to 13-fold. Together, these results provide evidence for up to 13-fold variation in CYP2E1 activity based solely on genetic diversity, which can translate in an up to 435-fold difference in formation of reactive metabolites. Thus, a small difference in baseline activity can be expected drive the risk phenotype. To our surprise, CYP2E1 activity did not correlate with protein adduct formation. In addition, QTL analyses did not reveal any association of protein adduct formation with certain genetic traits. Together these results show that BD uptake, distribution and metabolism is not limited by CYP2E1 activity and suggest that several other proteins are involved, such as other phase I and phase II or membrane transport proteins. Further, 60 inbred strains are insufficient to identify genetic loci that drive complex mechanism such as the toxicokinetics of carcinogens. In contrast, adduct formation in the relative small CC population was similar than reported for human exposure study of similar size, demonstrating that the complexity of populations can be investigated using the CC mouse model.

2257 A Multi-scale Stochastic Model Explains Zoned Cytochrome P450 Induction in the Liver Lobule

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2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD), one of the most potent environmental toxins, significantly induces cytochrome proteins, including Cyp1a1 and Cyp1a2, with a centrally localized spatial profile in the liver lobule. Besides toxin-induced zonal gene induction, metabolic functions in the liver lobule are also zonated along the central-portal axis. To investigate the roles of metabolic zonation in spatially restricted gene induction, we have developed a multi-scale, agent-based spatial model of a mouse liver lobule. The spatial location of hepatocytes in this model was calibrated from single-cell resolution imaging data. In each cell, a stochastic model was implemented to capture the switch-like Cyp1a1 induction by TCDD. The initial conditions for the model were determined from liver zonation experimental results. With this model, we investigated the role of the Wnt signaling pathway and the stochastic character of spatially-zonated Cyp1a1 gene induction. The results collected in this study reveal that endogenous Wnt signaling and activation of Cyp1a2, which binds and sequesters TCCD in the liver, together regulate the zonal hepatic induction of AhR target genes. Our model provides a more mechanistic understanding of low-dose toxic responses in the liver.

2258 Simulation of Macrophage Activation Dynamics under Various Microenvironment Signals Using an Agent-Based Modeling Approach


In this study we present a new multiscale Agent-Based Model (ABM) that spans molecular, cellular and tissue levels to provide a computational simulation framework aimed at reproducing and elucidating the dynamics of macrophage phenotypes under various complex activation signals, while considering system stochasticity and heterogeneity. Key factors in signaling cascades are included in the model, so that critical underlying regulatory controls influencing the activation process can be explored and quantified. Specifically, the model assumes that expression dynamics of pro- and anti-inflammatory cytokines are primarily regulated by activation of the following transcription factors: Nuclear factor-kappa B (NFkB), nuclear factor (erythroid-derived 2)-like 2 (Nrf2) signaling transducer of (STAT3) and STAT6. These factors were selected as representative controllers of transcriptional responses due to their essential role in inflammation. Activation of NFkB in macrophages induces transcription of pro-inflammatory cytokines, chemokines and other inflammatory mediators; the activity of NFKB is primarily modulated by the activity of its kinase (IKK) and its inhibitor (IκB) through the Toll-like receptor (TLR) signaling pathway. The activity of Nrf2 is mainly mediated by interactions with the Kelch-like ECH-associated protein 1 (Keap1) and activities of STAT3 and STAT6 are mainly mediated by the activity of Janus kinase 1 (JAK1), JAK3 and tyrosine kinase 2 (TYK2) following binding of anti-inflammatory cytokines to corresponding receptors. Crosstalk between the NFKB and Nrf2 pathways is also incorporated in the model. Model simulation results are presented and compared with available data from an in vitro system of mouse bone marrow-derived macrophages exposed to various doses of LPS/IFNγ and IL-4/IL-13. It is shown that our model has the ability to qualitatively reproduce dynamic patterns of macrophage phenotypes over time. A quantitative metric, i.e. a “macrophage activation (or polarization) ratio”, is defined to explore how cytokine signaling translates into polarization and to search for important regulatory components and model sensitivity analysis was performed to identify factors having large impacts on this metric. Supported by NIH grants E5050202, E5019584 and E5004738.

2259 Metabolome-Wide Association Study of Deployment to Balad, Iraq or Bagram, Afghanistan

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Concern about occupational exposures to military personnel and adverse health outcomes resulted in 2006 Department of Defense (DoD) Instruction 6490.03 requiring Military Services to perform comprehensive deployment health risk assessments that includes baseline, routine, and incident-related health surveillance and documentation of deployment-related exposures. To support this effort, we assembled a multidisciplinary research team to assess the utility of serum in the Department of Defense Serum Repository (DoDSR) for biomonitoring chemical exposures and identifying biomarkers associated with exposures. In the present study, we used high-resolution metabolomics (HRM) to identify metabolic changes in military personnel associated with deployment in Balad, Iraq, or Bagram, Afghanistan. The numbers in the current study consisted of paired pre- and post-deployment data for 373 individuals (n = 187 in Case group; n = 186 in Control group). HRM and bioinformatics were used to identify metabolic differences associated with deployment. The results show that differences at baseline (pre-deployment) between personnel deployed to Bagram compared to Balad or Controls included sex hormone and keratin sulfate metabolism. Differences associated with deployment to Balad included amino acid and lipid metabolism associated with inflammation and oxidative stress, and pathways linked to metabolic adaptation and repair. Difference associated with deployment to Bagram included lipid pathways linked to cell death and metabolism associated with innate immunity and keratin metabolism. Differences associated with deployment show that 26 of 271 environmental chemicals (ToxCast) differed in association with deployment to Balad, and 3 of these chemicals including trichlorfon metrifonate, dinitrofuram and ametryn clustered with metabolites that differed in association with deployment. In conclusion, metabolic differences in pre- and post-deployment are consistent with deployment-associated responses to air pollution and other environmental stressors.

2260 Neonatal Exposure to Environmental Chemicals BPA, BDE-99, and PCB Persistently Alters the Liver Transcriptome in Adult Mice

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Persistent environmental chemicals such as bisphenol A (BPA), polybrominated diphenyl ethers (PBDEs), and polychlorinated biphenyls (PCBs) are linked to many human diseases. It is increasingly recognized that there is a life-long impact on disease risk. The goal of the present study was to test our hypothesis that neonatal exposure to these chemicals persistently dysregu-
lates the transcriptome of the liver, which is the major organ for xenobiotic metabolism and nutrient homeostasis. Two-day-old male and female C57BL/6 mice were suprapelinguously exposed to corn oil, BPA (250μg/kg), BDE-99 (an enriched PBDE congener in humans, 57mg/kg), or the Fox River Mixture (an environmentally relevant PCB preparation, 30mg/kg), once daily for three days. RNA-Seq was conducted in livers from 5- and 60-day old mice. Data were analyzed using Hsats2, Cufflinks, Ingenuity Pathway Analysis (IPA), and the R package topGO. Neonatal exposure to all three chemicals persistently altered the expression of genes involved in the metabolism of drugs, carbohydrates, and lipids, as well as epigenetic modifications, with males being more susceptible than females. BPA and BDE-99 had more prominent effects compared to PCBs. Top predicted upstream regulators included the tumor suppressor p53 (persistently down-regulated by BPA in females), the sterol biosynthesis regulator Scap (persistently regulated by both BDE-99 and PCBs), as well as the nuclear receptors that regulate lipids (PPARs) and drug metabolism (CYPs). The long non-coding RNA (IncRNA) H19, linked to hepatocellular carcinoma, was persistently increased by BPA and BDE-99. Within the imprinted Dlk1-Dio3 cluster, which expresses genes in a parent-specific manner and is known to promote liver tumor, BPA and BDE-99 persistently increased the paternal allele-specific IncRNAs Meg3 and Rian, whereas protein-coding genes from the maternal allele remained silent. Our results demonstrate that neonatal exposure to these environmental chemicals persistently regulates the liver transcriptome in adulthood, possibly due to cross-talk between epigenetic reprogramming and nuclear receptors.

2261 Metabolome Wide Association Study of Occupational Exposure to Benzene
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Benzene is a recognized hematotoxin and leukemogen; however, its mechanisms of action in humans remain unclear. To provide insight into the processes underlying the relationship between benzene exposure and disease, we performed high-resolution metabolomics (HRM) profiling of plasma collected from a cross-sectional study of 33 healthy workers exposed to benzene (median 8-hr time-weighted average; 36 ppm), and 25 matched unexposed controls in Shanghai, China. Metabolic features associated with benzene were identified using a metabolome-wide association study (MWAS) framework that tested for the relationship between feature intensity and benzene exposure. Following correction for multiple testing, associated features were characterized for the presence of known and predicted benzene metabolites, and biological response by pathway enrichment. MWAS identified 478 mass spectral features associated with benzene exposure at FDR<20%. Comparison to a list of 13 known benzene metabolites and 86 metabolites predicted using a multi-component biotransformation algorithm showed five metabolites were detected, which included the known metabolites phenol and benzene dioxepoxide. Metabolic pathway enrichment identified 41 pathways associated with benzene exposure levels, with altered pathways including mitochondrial shuttle, fatty acid metabolism, sulfur amino acid metabolism, glycolysis, glutathione metabolism, and branched chain amino acid metabolism. These results, which suggest disruption to fatty acid uptake, energy metabolism and increased oxidative stress, point towards pathways related to insulin resistance and mitochondrial dysfunction, which has previously been linked to benzene exposure in animal models and human studies. In addition, fatty acid oxidation has been identified as a critical pathway for hematopoietic stem cells (HSCs) and controls stem cell self-renewal. Taken together, these results may suggest benzene exposure is associated with disruption of mitochondrial pathways, and provide a plausible mechanism underlying benzene-induced hematotoxicity in humans.

2262 Study of Hookah/Cigarette Constituents-Gene/Protein-Pathway-Disease Using Systems Biology Approach
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Hookah smoking is becoming increasingly popular worldwide. Recent epidemiological studies have indicated that hookah smoking is significantly associated with respiratory diseases, and may contain more harmful constituents than cigarette smoking. In addition, gene expression alterations and mechanisms of disease development affected by the constituents of hookah smoking are still unclear. To address these issues, we propose the novel experimental design and analyzing strategy to study the effects and mechanisms of hookah smoking and its constituents (e.g., nicotine, CO and PAH). Here, we performed large-scale profiling (i.e., NGS) at 2 and 24 hours after exposing the human lung epithelial cells (BEAS-2B) to five different conditions of the hookah smoking (i.e., control, charcoal only, charcoal with tobacco, charcoal with herbal, and electronic heater with tobacco). Systematic analyzing strategy was used to compare our results with public hookah and cigarette smoking datasets. Firstly, we identified 271, 313 and 198 differentially expressed genes (DEGs) (* test p < 0.05 and FC > 1.5) from our hookah set (charcoal with tobacco vs. control), the public hookah dataset and public cigarette datasets, respectively. Our result shows that more infection-related pathways (e.g., Salmonella infection; hypergeometric test p < 0.05) were identified from the hookah datasets than from the cigarette datasets, which might result from polluted hookah device or used contaminated water filters. Next, we identified individual constituent in hookah smoking by genome-wide comparison between tobacco and herbal datasets (the nicotine dataset) and identified 89 DEGs related to nicotine. Interestingly, at 24 hours after exposure, the cardiovascular system development, dopaminergic synapse and fatty acid metabolism pathways were activated by nicotine. In addition, 645 DEGs are related to charcoal in comparison to electronic combustion (the CO and PAH dataset). The inflammation and diabetes mellitus related genes and pathways were activated at 2 and 24 hr after exposure in the CO and PAH dataset. Overall, our results show that hookah smoking may have higher risk in bacterial infection than cigarette smoking and link the hookah constituents to diseases. These results imply that frequent hookah smoking is more likely to harm people than cigarette smoking.

2263 Serum Metabolomics Reveals That Gut Microbiome Perturbation Mediates Arsenic Toxicity in Mice
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Arsenic contamination in drinking water has been a global health concern over many years. Except for well-recognized carcinogenic effects, numerous studies have also linked arsenic exposure with a variety of chronic diseases such as diabetes, neurological effects, and cardiovascular diseases, although the etiology underlying is still unclear. Recently, increasing evidence have indicated that gut microbiome is an important risk factor in modulating the development of diseases. This study aims to investigate the role of gut microbiome perturbation in arsenic-induced diseases by coupling a high-resolution mass spectrometry-based global metabolomics approach and an animal model with altered gut microbiome induced by bacterial infection. Serum metabolic profiling revealed that when gut microbiome homeostasis was perturbed, both the number and regulation pattern of the metabolites with significant differences induced by arsenic exposure changed dramatically. Specifically, the metabolic pathways related with fatty acids, phospholipids, sphingolipids, cholesterol, and tryptophan changed dramatically, and these pathways were not or were less disrupted when gut microbiome stays normal. Our study suggested that gut microbiome perturbation can exacerbate or cause the metabolic disruption induced by arsenic exposure in mice.

2264 Use of an Alternative Vehicle in the Human Cell Line Activation Test (h-CLAT) to Broaden the Utility of the Test to Detect Dermal Sensitizers
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The human Cell Line Activation Test (h-CLAT) was developed as an in vitro method of discriminating skin sensitizers from non-sensitizers and was promulgated by OECD as a testing guideline (OECD TG 442E) in the spirit of advancing the 3Rs (Replacement, Reduction, and Refinement). As part of an Integrated Approach to Testing and Assessment, this test method addresses the third key event of the skin sensitization Adverse Outcome Pathway by measuring the selective induction of the surface markers CD54 and CD86 in the human monocyte leukemia cell line THP-1, which functions as a dendritic (Langerhans) cell surrogate. This guideline test is hindered by the sanctioning of only two currently validated vehicles (DMISO and saline), precluding in vitro sensitization testing of a wide range of insoluble chemicals. One such insoluble test chemical, Tetrakis (2-ethyl butyl) Orthocarbonate, was evaluated in the h-CLAT using an alternative vehicle (i.e., 100% ethanol) in an effort to broaden
the utility of the test. Known reference chemicals representing strong, and moderate sensitizers such as 2,4-dinitrochlorobenzene (DNBC) and mercaptotobenzothiazole (MBT) (used as positive controls), as well as a non-sensitizer such as isopropanol (used as a negative control), were also included to evaluate the utility of ethanol as an alternative h-CLAT vehicle. In two replicate runs, DNBC and MBT induced positive responses for CD54 (RFI >200%) and CD86 (RFI >150%) expression as expected, whereas isopropanol resulted in negative responses for both cell-surface markers. Solvent-only controls confirmed that 100% ethanol itself did not cause cytotoxicity or produce positive responses in either CD54 or CD86. The test chemical did not induce positive responses for CD54 or CD86 in the modified assay; however, due to other limiting physical-chemical properties of such a test as LOG kow <3.5, the negative test result did not inform the ultimate determination of hazard classification. This work demonstrates that 100% ethanol is a suitable alternative solvent which may expand the applicability domain of the h-CLAT.

2265 Proteomic and Bioinformatic Analyses for the Identification of Proteins with Low Allergenic Potential for Use in Hazard Assessment

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Use of botanicals and natural substances in consumer products has increased in recent years. Such extracts can contain protein that may theoretically represent a potential risk of IgE-mediated allergy. No method has yet been generally accepted or validated for assessment of the allergenic potential of proteins. For development of suitable methods the datasets of allergenic and non-allergenic (or low allergenic) proteins are required that can be compared, respectively, as positive and negative controls. However, data are unavailable on proteins that lack or have low allergenic potential. Here, low allergenic potential proteins are identified based upon the assumption that proteins with established human exposure, but with a lack of an association with allergy, possess low allergenic potential. Proteins were extracted from sources considered to have low allergenic potential (corn, potato, spinach, rice and tomatoes) as well as higher allergenic potential (wheat) regarding the most common allergenic foods. Proteins were identified and semi-quantified by label-free proteomics analysis conducted using mass spectrometry. Predicted allergenicity was determined using AllerCatPro (https://allercatpro.bii.a-star.edu.sg/). This in silico tool compares proteins on a sequence and 3D structure level with a dataset of >4,000 protein allergens and performs with 84% accuracy to predict allergens versus non-allergens. In summary, 9070 proteins were identified and semi-quantified from six protein sources. Within the top 10% of the most abundant proteins identified, 178 characterized proteins were found with no evidence for allergenicity predicted by AllerCatPro and were considered to have low allergenic potential. This panel of low allergenic potential proteins provides a pragmatic approach to aid the development of alternative methods for more robust testing strategies to distinguish between proteins of high and low allergenic potential to assess the risk of novel proteins of high and low allergenic potential to assess the risk of novel proteins.

2266 Efferocytosis of cSiO2-Induced Cell Corpses by the Max Planck Institute Alveolar Macrophage Model Is Potentiated by the Omega-3 Docosahexaenoic Acid (DHA)

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Occupational exposure to crystalline silica (cSiO2) has been ethnologically linked to autoimmune disease. We previously demonstrated that intranasal instillation of lupus-prone mice with cSiO2 markedly accelerates systemic autoimmunity and glomerulonephritis by promoting pulmonary ectopic lymphoid neogenesis. Rapidly, all of these effects were prevented when mice were fed with omega-3 docosahexaenoic acid (DHA) at translationally relevant concentrations. Alveolar macrophages (AMph) rapidly phagocytose cSiO2, ultimately resulting in their death. Inefficient removal of resultant cell corpses via a phagocytic process known as efferocytosis, can increase a host’s exposure to autophagosomes thereby contributing loss of tolerance and autoimmunity. Here we determined how DHA influences the ability of cSiO2-generated cell corpses to be recognized during efferocytosis and/or the efferocytic capacity of AMph. ASC-transfected RAW 264.7 cells (target) were pre-incubated with or without 25 µM DHA, labelled with CFSC, and their death induced by incubation with cSiO2. Resultant cell corpses were then incubated for up to 16 h with CytoTox™-labelled Max Planck Institute (MPI) cells (effector), an alveolar macrophage surrogate, that were also pre-incubated with or without DHA (25 µM). Amnis® imaging flow cytometry revealed that maximum efferocytosis occurred at 8 h with the highest clearance of death cell corpses when target cells were pre-incubated with DHA. Since the recognition of dying cells during efferocytosis depends on the externalization of phosphatidylserine (PS) on the cell membrane, total PS along and its externalization were measured in target cells after 24 h pre-incubation with/without 25 µM DHA. DHA pre-incubation not only increased total PS content but also increased PS externalization in cSiO2-induced dying target cells 4, 6 and 8 h after addition of the particles. Overall, this study suggests that DHA supplementation enhanced PS externalization during cSiO2-induced cell death and that this corresponded to functional improvement in recognition by AMph-like cells and consequent engulfment of cell corpses. These findings might explain, in part, reduced cSiO2-stimulated autoimmune nephritis observed in lupus-prone mice consuming DHA. Supported by NIHES grant ES027353, Lupus Foundation, and Dr. Robert and Carol Diebel Family Endowment.

2267 Impact of Trichloroethene Metabolite Dichloroacetyl Chloride on Nrf2/Keap/HO-1 Axis: Potential Role in Autoimmunity

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Our previous studies have shown that trichloroethylene (TCE) and one of its reactive metabolites dichloroacetyl chloride (DCAC) are associated with the induction of autoimmunity response in MRL+/+ mice. Our studies also show that oxidative stress in TCE/DCAC-mediated autoimmunity. Nuclear factor (erythroid-derived 2)-like2 (Nrf2) is an antioxidant stress-related transcription factor that binds to antioxidant responsive element (ARE). The Nrf2-ARE pathways protect against reactive oxygen species by regulating a great number of cytoprotective and antioxidant gene expression. We used immortalized murine Kupffer cells (KCs) and human Jurkat T cells that were treated with an antioxidant and Nrf2-activator tert-butyldihydroquinone (tBHQ) one hour prior to incubation with 5mM of DCAC for 24 hours. Treatment with DCAC significantly inhibited Nrf2 and Nrf2 target genes HO-1 and Keap-1 mRNA expression, but elevated phospho-Nrf2 protein level. Furthermore, DCAC-mediated inhibition was associated with increased mRNA expression of Nrf2 downstream inflammatory target NF-kB (p65) and TNF-α mRNA. The results show that DCAC-mediated impairment of Nrf2 regulation could be critical in generating a pro-inflammatory response and could play a crucial role in the induction of DCAC-mediated autoimmunity. Further studies are required to fully understand the contribution of Nrf2/Keap/HO-1 axis in TCE/DCAC-mediated autoimmune response. Supported by NIH R01 grants E5026887 and E5016302.

2268 Contribution of Cytochrome P450 2E1 in Trichloroethene-Mediated Autoimmunity: Association with Oxidative Stress and Nrf2 Pathway

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Reactive trichloroethene (TCE) metabolites and oxidative stress are involved in TCE-mediated autoimmunity, as evident from findings of our previous studies in MRL+/+ mice. However, molecular mechanisms underlying TCE-mediated autoimmunity remain largely unknown. Cytochrome P450 2E1 (CYP2E1), the major enzyme responsible for TCE metabolism, could contribute to TCE-mediated toxic response through free radical generation. The current study was, therefore, aimed to further evaluate the significance of TCE metabolism leading to oxidative stress and autoimmune response by using MRL+/+ mice that lack CYP2E1. The Cyp2e1-null MRL+/+ mice were generated by backcrossing Cyp2e1-null mice (B6N:129S4-Cyp2e1) to MRL+/+ mice. Female MRL+/+ and Cyp2e1-null MRL+/+ mice were given TCE (10 mmol/kg, i.p., every fourth day) for 6 weeks; their respective controls received corn oil only. TCE treatment in MRL+/+ mice induced oxidative stress, evident from significantly increased serum anti-malondialdehyde (MDA)- and anti-4-hydroxynonenal (HNE)-adducts antibodies and reduced liver GSH. TCE treatment also modulated Nrf2 pathway with decreased Nrf2, HO-1 and elevated NF-kB (p65) expression in the liver. TCE exposure also led to increases in serum anti-tdN nucleic acids (ANA) and anti-double stranded DNA antibodies (anti-dsDNA). Although TCE treatment in Cyp2e1-null MRL+/+ mice also led to increases in serum anti-MDA/HNE-adducts antibodies and changes in liver...
GSH, Nrf2, HO-1 and NF-κB along with increases in serum ANA, anti-dsDNA, the changes in the oxidative stress and autoimmunity markers in these mice were less pronounced compared to that in MRL+/+ mice. These findings support the contribution of CYP2E1-mediated TCE metabolism in autoimmune response and an important role of Nrf2 pathway in TCE-mediated autoimmunity. Supported by NIH ES0670 and ES026887.

2269  
Modulation of Trichloroethene-Mediated Hepatic Inflammamsonme Activation and Immune Dysregulation by Antioxidant N-Acetylcysteine

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Trichloroethene (TCE) exposure is associated with the development of various autoimmune diseases, including autoimmune hepatitis (Aih) and systemic lupus erythematosus (SLE), potentially through the generation of excessive reactive oxygen and nitrogen species (RONS; oxidative stress). However, the mechanisms by which oxidative stress contributes to these TCE-mediated autoimmune diseases are not fully understood, and are the focus of current investigation. Female MRL+/+ mice were treated with TCE and along with or without antioxidant N-acetylcysteine (NAC) for 6 weeks (TCE, 10 mmol/kg, i.p., every 4th day; NAC, 250 mg/kg/day via drinking water). TCE exposure resulted in activation of hepatic inflammasomes (NLRP3 and caspase-1) and up-regulation of pro-inflammatory cytokine IL-1β, and these changes were significantly reduced following NAC supplementation. TCE treatment also led to increased hepatic lymphocyte infiltration, including cytotoxic T cells (CD3+CD8+CD44+CD62L−), dendritic cells (CD11b+CD11c), NK cells (CD49b+), and activation of both hepatic and splenic B cells (B220+GL7+ cells). Furthermore, TCE treatment resulted in a reduction of the Treg to Th17 cell ratio. Interestingly, NAC-mediated but hepatic and splenic immune cells was effectively attenuated by NAC. More importantly, TCE exposure also induced an increase in anti-nuclear antibodies (ANA) in the serum, which was also significantly reduced following NAC supplementation. Taken together, our findings provide evidence for TCE-mediated inflamma- somes activation of various immune cells, and skewed balance of Treg and Th17 cells in the liver. The attenuation of TCE-mediated hepatic immune responses by NAC strongly supports the role of oxidative stress in TCE-mediated autoimmunity. These novel findings could help in designing therapeutic strategies for such autoimmune diseases. Supported by NIH R01 grants ES062887 and ES016302.

2270  
Percent n-3 in Highly Unsaturated Fatty Acids (HUFAs) Is Predictive of Disease Outcomes in Environmental Toxicant-Triggered Autoimmunity


Increased tissue content of n-3 highly unsaturated fatty acids (HUFAs, containing ≥20 carbons with ≥3 double bonds) at the expense of n-6 HUFAs may protect against environmental toxicant-triggered autoimmunity. Our laboratory currently uses the female NZBWF1 mouse to understand how consumption of docosahexaenoic acid (DHA, 22:6 n-3), a major HUFA in fish oil, can be used to prevent or treat lupus induced by crystalline silica (cSiO2), a known environmental trigger of human autoimmune disease. Previously, we found that supplementation with DHA dose-dependently decreased the magnitude of several pathogenesis endpoints of cSiO2-triggered lupus including pulmonary lymphocyte infiltration, B and T cell-containing ectopic lymphoid structures (ELS) in lung, and pulmonary and systemic inflammation and cytokines. Here, we related these cSiO2-induced disease endpoints to the %n-3 in HUFA in the red blood cells (RBC), lung, spleen, and kidney. The %n-3 in HUFA measures n-3 HUFA as percent of total HUFA rather than percent of total fatty acids, thereby decreasing variation by limiting analysis to the HUFA pool. Increased %n-3 in HIFA was associated with decreased autoim- mune biomarkers, such as anti-dsDNA IgG antibodies (R2=0.38, p=2E-5) and B-cell activating factor (R2=0.36, p=4E-4). Increased B-cell %n-3 in HUFA was also associated with less B and T cell infiltration in the lungs (R2=0.46, p=2E-6 and R2=0.49, p=1E-6, respectively), and decreased levels of pro-inflammatory cytokine TNF-α (R2=0.41, p=1E-5) and chemokine MCP-1 (R2=0.38, p=2E-5). Similar associations were found between these endpoints and %n-3 in HUFA in the lung, spleen, and kidney. These findings demonstrate that dis- ease phenotypes induced by cSiO2 exposure strongly correlate with %n-3 in HUFA in RBC and other tissues. This biomarker might therefore be useful in a precision medicine approach exploiting n-3 HUFA supplementation to prevent and/or treat lupus and other autoimmune diseases triggered by envi- ronmental toxins. Supported by NIH grant ES02753, Lupus Foundation grant 362470, the Dr. Robert and Carol Diebel Family Endowment, and the Institute for Integrative Toxicology NIHES Training Grant T32ES007255.

2271  
In Situ Mapping of the Reactivity of Chemical Sensitizers in Reconstructed Human Epidermis Using High-Resolution Magic Angle Spinning (HRMAS) NMR Technique

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Allergic contact dermatitis is a reaction of the immune system resulting from skin sensitization to an exogenous hazardous chemical. The prevalence of this disease (15-20%) has increased and, as there is no treatment other than symp- tomatic, the prevention relies on the evaluation of the sensitizing potency of chemicals prior to their introduction on the market. One of the alternatives to animal methods for risk assessment of chemicals is by measuring their reac- tivity towards epidermal proteins. So far, this was approached using isolated small reactive peptides in solution but these tests are far from reflecting the complex chemistry taking place in a living epidermis. With the aim of replac- ing animal tests while maintaining the most similar reaction conditions to the human epidermis, we have developed a new method based on the use of HRMAS NMR to monitor in situ the reactions of carbon 13 substituted chemi- cal sensitizers with nucleophilic amino acids in reconstructed human epidermis (RHE). RHE were treated with chemicals and spectra were acquired using HRMAS NMR and then analyzed using HRMAS NMR quantitave sequences. We were thus able to study the reactivity profile of chemicals in situ as a function of exposure time and dose. First, both MMS and CA were found to react rapidly in RHE with a maximum concentration of add- ucts between 5h and 8 h for MMS but already at 30 min for CA. Second, MMS was found to react mainly with Cys/His but also with Lys and Glu minor extent with Glu/Asp, Lys and Met, while CA was reacting only with Cys and Lys. Third, dose/ response studies have shown that the reactivity with Cys was easily saturable and therefore associated with GSH reaction. Fourth, the metabolism of CA to cinnamic acid and cinnamyl alcohol was observed and quantified. Therefore, using quantitative HRMAS NMR sequences and RHE we were able to study the reactivity of two sensitizing chemicals as a function of exposure time and dose and to monitor in situ broader aspects of reactivity like detoxication and metabolism processes which are not covered when using peptides in solu- tion. 

2272  
Å6-Tetrahydrocannabinol Suppression of Monocyte-Mediated Astrocyte Production of MCP-1 and IL-6 in a Co-Culture Stimulated with TLR7 Agonist

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Migration of monocytes to the brain has been implicated as a key event in chronic neuroinflammation and in the etiology of several central nervous sys- tem diseases including HIV-associated neurocognitive disorder. In the brain, activated monocytes have the potential to cause neuronal injury through pro-inflammatory interactions with astrocytes. This includes inducing as- trocyte secretion of cytokines and chemokines, which promotes a positive feedback loop of neuroinflammation. Cannabis is widely used by HIV-infected individuals with a prevalence of 20-37% in the United States and Canada. Å6- tetrahydrocannabinol (THC) and cannabidiol (CBD), two major constituents of cannabis, are known to have immune suppressive and anti-inflammatory properties. Previously, we developed a human co-culture system that demon- strates that monocytes, though secretion of IL-1β, promote astrocyte produc- tion of MCP-1 and IL-6 in response to toll-like receptor 7 (TLR7) stimulation (mimic HIV ssRNA). The objective of this study was to determine the effect of THC and CBD on monocyte-mediated astrocyte inflammation when stimu- lated through TLR7. THC treatment of the TLR7-stimulated co-culture resulted in decreased astrocyte production of MCP-1 and IL-6, while CBD increased IL-6 and had no effect on MCP-1. With the use of monocyte and astrocyte monocolonies, THC and CBD targeted both cell types. Interestingly, THC and CBD were both shown to decrease IL-6 and MCP-1 in astrocyte monoclonies, which for THC, is consistent with the co-culture observation. However, di- totoxicological effects were observed with CBD, as there was augmentation in the co-culture and suppression in the astrocyte monoculture. These findings were explained when THC and CBD were shown to suppress and augment monocyte production of IL-18, respectively. Furthermore, the CBD-mediated augmentation of monocyte-derived IL-18 superseded the direct CBD sup- pression on astrocytes. Collectively, this study demonstrates that THC but not 2019 SOT Annual Meeting
CBD, suppresses monocyte-provoked astrocyte inflammatory responses. In summary, cannabis use (high THC/low CBD) may decelerate monocyte processes that are implicated in HIV-associated neuroinflammation.

**2273** Resveratrol-Induced FoxP3-Regulatory T Cell Subset, Th3, Alleviates Acute Lung Injury Induced by Staphylococcal Enterotoxin-B (SEB)  
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It is well known that the intake of food enriched with resveratrol (RES), 3,5,4’-trihydroxy-trans-stilbene, is useful in protection or treatment of diseases such as diabetes and cancer. We have shown previously that resveratrol treatment led to survival of C3H mice from fatality following exposure to dual dose of Staphylococcal enterotoxin B (SEB) via activation of TGF-β signaling in T regulatory cells of CD4+ FoxP3+ lineage. In the current study, we investigated the role of RES in boosting the anti-inflammatory effects via induction of T-helper3 cells (Th3). To this end, C3H/HeJ mice were pretreated orally with 100 mg/kg of RES, 24 hrs and 90 minutes before induction of acute lung inflammation by SEB, a CDC select agent of bioterrorism. Infiltrating cells from broncho-alveolar fluid lavage (BALF) and spleenocytes were harvested to measure biological parameters. In addition, in vitro polarization of spleenocytes pretreated with 50 µM RES or with the tryptophan metabolite, kynurenine (Kyn) derived from RES-treated mice was carried out following 1µg/ml SEB activation for 48 hrs. Our findings revealed increase in Th3 population in vivo and in vitro by flow cytometry particularly IL10 expressing cells in RES-treated group. Furthermore, protein quantification by ELISA showed increase in IL10 levels in BALF and plasma of RES-treated mice as well as in cell culture supernatant of RES- and Kyn-treated groups. In addition, Th1 and Th17 cell populations and their related cytokines were reduced significantly in lungs, sera and cultured cells treated with RES. PCR analysis showed significant elevation in the expression of Kyn mRNA in lung infiltrating cells and alveolar epithelial cells isolated from mice treated with RES, while STAT1 mRNA expression was significantly ablated in lung infiltrating mononuclear cells. Immunofluorescence investigation showed significant increase in the regulatory protein, MUC5b, which is involved in mucociliary clearance mechanism in respiratory system of RES-treated mice. Together, RES treatment induced increase in Th3 population which led to a decrease in Th1 and Th17 responses following SEB exposure. Supported by NIH P01AT003961, P20GM103641, R01AI219788 and R01AI219347.

**2274** Protective Effects of Sodium Butyrate Resulted from Reconstruction of Altered Gut Microbiota Mediated by SEB-Induced Acute Lung Injury  
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Inhalation of Staphylococcal Enterotoxin B (SEB) is known to induce acute lung injury (ALI). Sodium butyrate has been shown to have anti-inflammatory effect in some studies. In the current study, we investigated the role of sodium butyrate in attenuating ALI. Thus, a dual-dose of SEB was given to C3H/HeJ mice, which were then treated either with vehicle or butyric acid. SEB-administration caused ALI and 100% mortality within 5 days, while all butyrate-treated mice survived and suppressed the inflammation in the lungs by increasing anti-inflammatory cells including T regulatory cell lineage and myeloid derived suppressor cells. Moreover, we investigated the regulatory genes and we found that sodium butyrate activates PPAR-gamma signaling pathway and Nos1, IL10 and decreases IFN-gamma. Furthermore, colon microbiota was collected and 16S rRNA sequencing was performed. The data were analyzed to determine the alpha and beta diversity. The major phylum was Firmicutes, class Clostridia and order was Clostridiales to the level of genus Ruminococcus in the colon of sodium butyrate-treated SEB group. Further, mouse transcriptome array shows decrease in genes of TNF and chemokines ccl12, ccl2, ccl26, and cxcr2, besides increasing claudin2, claudin34, defenses-alpha and defenses-beta. Together, our data suggests that butyrate attenuates SEB-induced mortality and ALI by reconstructing altered microbiota. Supported by NIH grants P01AT003961, R01AI219347, R01AI219788 and P20GM103641 to PN and MN, and MohESR fellowship for AKM.

**2275** TCDD Alters Microbiome and Induces Myeloid-Derived Suppressor Cells That Inhibit T Cell Activation by Depleting Cysteine  
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Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of cells that are defined by their myeloid origin, immature state, and ability to potently suppress T-cell and B-cell responses. Murine MDSCs are characterized by the expression of CD11b and GR1 cell markers and can be subdivided into two groups, Monocytic and Granulocytic based on the expression of Ly6C and Ly6G molecules. Previously, we found that one persistent environmental pollutant that induces MDSCs and MDSC subset expansion was 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). In the current study, we observed that injecting C57BL/6 mice with 10µg/kg TCDD alters gut microbiome by increasing of Lactobacillus abundance when compared to vehicle after three days. Increase in Lactobacillus is related to T-reg and MDSC induction. After fecal transplantation, we found mice that received feces from TCDD-treated mice have higher peritoneal MDSCs and MDSCs subset compared to mice that received feces from vehicle-treated mice. TCDD-induced MDSCs and Monocytic MDSCs led to reduction in splenic IL-17 and IFN-γ levels of these mice. Furthermore, 16S rRNA analysis of gut microbiota reveals reduction in cysteine metabolism pathway in the microbiome of treated mice. It is known that MDSCs block T cell activation by sequestering cystine and limiting the availability of cysteine. Our results from 3H-thymidine assay shows TCDD-induced MDSCs inhibit ConA-activated T cell activation when cultured with MDSCs whereas culturing with cysteine enhances T cell activation. Also, we found that TCDD-induced MDSCs have less ASC expression, neutral amino acid transporter that is important to export cysteine as well as less cystathionase, which converts methionine to cysteine when compared to vehicle. In summary, our data shows TCDD alters microbiome by increasing Lactobacillus enrichment that induced MDSCs. MDSCs suppress T cell activation through depleting cysteine in the environment. Supported by NIH P01AI039361, P20GM103641, R01AI219788, R01AI219347 and MOHESR/Iraq.

**2276** Immunomodulatory Activity of N-Butylbenzenesulfonamide in Female B6C3F1/N Mice  
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N-Butylbenzenesulfonamide (NBBS) is a high production volume plasticizer used in polyamide commercial and consumer products. NBBS has been detected in environmental water samples, and in leachate from polyamide cooking utensils, making oral exposure the likely route for human exposure. Distribution studies in rodents demonstrated that NBBS was rapidly absorbed into tissues, and eliminated rapidly, with little indication of accumulation. NBBS is reported to induce alterations in hematological parameters, and androgen and progesterone receptor mediated transactivation in adult rodents, and to adversely affect implantation and pregnancy in gestation studies. However, little is known regarding the impact of NBBS on the immune system. The objective of this study was to evaluate the potential immunomodulatory effects of NBBS in adult female B6C3F1/N mice, using a standard panel of innate, cell-mediated, and humoral-mediated immunity endpoints. Mice were exposed to 0-5000 ppm NBBS by dosed feeding for 28 days. Under the conditions of the study, NBBS exposure had little or no effect on body and organ weights, hematology, relative or absolute numbers of lymphocyte sub-populations in the spleen, or histopathology of lymphoid or selected non-lymphoid organs. In contrast to the lack of effects on structural and cellular immune functions, androgenic and progesterone receptor mediated transactivation in adult rodents, and to adversely affect implantation and pregnancy in gestation studies. However, little is known regarding the impact of NBBS on the immune system. The objective of this study was to evaluate the potential immunomodulatory effects of NBBS in adult female B6C3F1/N mice, using a standard panel of innate, cell-mediated, and humoral-mediated immunity endpoints. Mice were exposed to 0-5000 ppm NBBS by dosed feeding for 28 days. Under the conditions of the study, NBBS exposure had little or no effect on body and organ weights, hematology, relative or absolute numbers of lymphocyte sub-populations in the spleen, or histopathology of lymphoid or selected non-lymphoid organs. In contrast to the lack of effects on structural and cellular immune functions, androgenic and progesterone receptor mediated transactivation in adult rodents, and to adversely affect implantation and pregnancy in gestation studies. However, little is known regarding the impact of NBBS on the immune system. The objective of this study was to evaluate the potential immunomodulatory effects of NBBS in adult female B6C3F1/N mice, using a standard panel of innate, cell-mediated, and humoral-mediated immunity endpoints. Mice were exposed to 0-5000 ppm NBBS by dosed feeding for 28 days. Under the conditions of the study, NBBS exposure had little or no effect on body and organ weights, hematology, relative or absolute numbers of lymphocyte sub-populations in the spleen, or histopathology of lymphoid or selected non-lymphoid organs. In contrast to the lack of effects on structural and cellular immune functions, androgenic and progesterone receptor mediated transactivation in adult rodents, and to adversely affect implantation and pregnancy in gestation studies.

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Alterations in the Mouse Skin and Gut Microbiome following Dermal Exposure to the Antimicrobial Chemical Triclosan

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It is increasingly being recognized that the microbiome plays an important role in human health. Dysbiosis of the microbiome has been shown to alter immune responses and has been associated with increased risk of allergic disease. Triclosan is an antimicrobial chemical used in the healthcare field as a high level disinfectant. In humans, triclosan exposure has been associated with an increase in food and aeroallergy and asthma exacerbation. Although not directly sensitizing, dermal exposure to triclosan has been shown to augment allergic responses to experimental allergens in mouse models. However, the impact of dermal exposure to antimicrobials, such as triclosan, on the microbiome is unknown. This study investigated the impact of dermal exposure to triclosan on the skin and gut microbiome in mice. Mice were dermally exposed to 2–3% triclosan or acetone vehicle control for either 7 or 28 consecutive days. Swabs were used to collect skin commensal bacteria prior to exposure and over the course of the exposure period and fecal pellets were collected following the last triclosan exposure to assess gut commensal bacteria. Following bacterial DNA extraction from skin swabs and fecal pellets, composition of the skin and gut microbiota was determined by 16S ribosomal RNA sequencing. Sequences were grouped into operational taxonomic units and given taxonomic assignments. Analysis of changes in relative abundance identified decreased Proteobacteria and increased Firmicutes in the triclosan exposed group compared to the vehicle control. The skin and gut diverged on the class taxonomic level; Clostridia increased in skin samples, whereas Bacilli increased in the fecal pellet samples. Within the class of Clostridia, Lachnospiraceae and Ruminococcaceae were both increased in relative abundance in the skin swab samples. Lactobacillaceae within the class Bacilli was increased in abundance in the gut. Taken together, dermal exposure to triclosan altered the composition of commensal bacteria in both the skin and gut of mice, suggesting that triclosan can induce dysbiosis of the microbiome and this may contribute to the observed alternations in immune function.

Investigation on the Possible Role of microRNAs in the Regulation of Chemical Allergen Potency

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Allergic contact dermatitis (ACD) is an immunological mediated inflammatory disease and is one of the most common occupational diseases in industrialized countries. ACD is a T-cell mediated skin inflammation caused by repeated skin exposure to low molecular weight chemicals. Chemical allergy is of considerable importance to the toxicologist, who has the responsibility of identifying and characterizing the allergenic potential of chemicals. While incredible progresses have been made in the development of non-animal tests, currently it is not possible to estimate the sensitizing potency of chemical allergens. Using THP-1 cell line, a model for primary human monocytes, and assuming that the extent of chemical allergen-induced dendritic cells activation/maturity and lifespan may drive the quality and magnitude of T cell activation, we have conducted a study using allergens of different potency to verify this hypothesis. Up-regulation of CD80, CD86 and HLA-DR, and the release of several cytokines were evaluated in THP-1 cells. Results indicate that extreme allergens of different potency differently activate DCs, with extreme allergen induction/maturation and lifespan may drive the quality and magnitude of T cell activation. We have conducted a study using allergens of different potency to verify this hypothesis. Up-regulation of CD80, CD86 and HLA-DR, and the release of several cytokines were evaluated in THP-1 cells. Results indicate that extreme allergens induce a more rapid CD86 up-regulation (24h) compared to the moderate and weak allergens. Furthermore, HLA-DR was up regulated at 72h only by the extreme sensitizers. Overall, results suggest that allergens of different potency differently activate DCs, with extreme allergen inducing a higher degree of maturation compared to moderate and weak allergens. Based on these results, we moved to the analysis of miRNAs expression in response to chemical allergens as valuable explanation to understand allergenic potency. MVs release by immune system cells may be induced by soluble agonists or in response to physical or chemical stress. MicroRNAs are non-coding RNA molecules that regulate gene expression at the post-transcriptional level. Recent findings indicate that miRNAs contained in MVs may determine reprogramming of gene expression in target cells. Although ACD has been studied extensively, there are few studies conducted to investigate miRNA expression. Using miScript miRNA PCR Array (Qiagen) we identified few miRNAs involved in ACD. These include let-7, miR-142 and miR-155. We are currently investigating the possible correlation between the miRNA expression observed with the screening and the effective potency of selected contact allergens tested. Acknowledgements: This study was supported by funding from Colgate-Palmolive Grant for Alternative Research (SOT Award 2017–2018).

The Effects of Pristine and Carboxylated Multiwalled Carbon Nanotubes on Phagocytic Function of Macrophages

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The global production and applications of multi-walled carbon nanotubes (MWCNTs) have increased in recent years despite evidence that MWCNTs cause pulmonary fibrosis in lab animals that may lead to mesothelioma. Studies to understand the pathological mechanisms of MWCNTs often 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 ~100X more carboxylated MWCNTs (C-MWCNTs) than pristine MWCNTs (P-MWCNTs). Also, Class A scavenger receptors expressed in macrophages may be involved in the selective accumulation of C-MWCNTs (Wang et al., Nanotoxicology, 2018, DOI: 10.1080/17435390.2018.1472309). To investigate the potential impacts of accumulated C-MWCNTs and P-MWCNTs on phagocytic function of macrophages, this study focused on two approaches: 1. Mouse macrophage RAW 264.7 cells were pre-exposed to C-MWCNTs or P-MWCNTs at 37° C for 2h and 24h, washed and challenged with 1 μm fluorescent polystyrene beads to monitor the phagocytic activity of the cells. This was done quantitatively through flow cytometry and qualitatively using confocal fluorescence microscopy. The fluorescent intensities of the cells treated with either C-MWCNTs or P-MWCNTs were compared to that of the control cells. The results demonstrated that 24h exposure to C-MWCNTs reduced phagocytosis of beads by 30-40% versus the control while exposure to P-MWCNTs did not suggest that 24h pre-exposure to C MWCNTs impaired phagocytic function. 2. To further investigate the impact of C MWCNTs on macrophages, the second approach will employ GFP expressing E. coli as a phagocytic marker. Cells treated with C-MWCNTs or P-MWCNTs will be washed and then challenged with E. coli. The phagocytosed bacteria will be detected using confocal fluorescence microscopy. The difference in bacteria uptake with and without pre-exposure to either C-MWCNTs or P-MWCNTs will be quantitatively measured using flow cytometry. The approaches developed in this study will facilitate the assessment of potential impacts of various MWCNTs on macrophages' defensive function to phagocytose pathogenic bacteria.

Development of a Zebrafish Larvae-Screening Assay to Identify Compounds with Immunotoxicity and Anti-inflammatory Activity


Zebrafish is a unique model for pharmacological manipulation of the innate and adaptive immune response. They are small and permeable to many small compounds and there are several transgenic lines available to visualize cells from the innate immunity (neutrophils and macrophages) and adaptive immunity (B and T cells). Taking advantage of zebrafish embryo transparency, we can test the toxicity of pharmacological, agrochemical and cosmetic compounds to the immune system by quantifying these cell populations. Additionally, this model can also be used to identify new anti-inflammatory compounds by following leukocyte recruitment to inflammation induced by sterile tissue injury. We have developed an assay in zebrafish larvae to detect compounds with specific toxicity for the immune system and to screen and identify new anti-inflammatory drugs. For these purposes two transgenic lines have been used: neutrophil-specific Tg (mpx:GFP)/114 and macrophage specific Tg (mpeg:mcherry). Different reference compounds with known anti-inflammatory effect were chosen and their doses selected after an MTC (Maximum Tolerated Concentration) assay carried out in 3 days post fertilization (dpf) embryos (when the innate immune system is already in place). To determine compounds toxicity at the innate immune system level, embryos were exposed to fresh dose for 48 h and the population of neutrophils and macrophages was quantified by fluorescence microscopy. Inflammation was induced by sterile injury of the tail fin and neutrophil recruitment to the wound site was assessed at 4, 6 and 12 h post injury in the presence of reference compounds using a partially automated platform. The ability of the compounds to suppress the expression of inflammatory genes (Il1b, Inf-a) was also evaluated by quantitative PCR. This zebrafish assay shows to be a cost-effective assay over mammalian models for the identification of new anti-inflammatory compounds and there are several transgenic lines available to visualize cells from the innate immunity (neutrophils and macrophages) and adaptive immunity (B and T cells). Taking advantage of zebrafish embryo transparency, we can test the toxicity of pharmacological, agrochemical and cosmetic compounds to the immune system by quantifying these cell populations. Additionally, this model can also be used to identify new anti-inflammatory compounds by following leukocyte recruitment to inflammation induced by sterile tissue injury. We have developed an assay in zebrafish larvae to detect compounds with specific toxicity for the immune system and to screen and identify new anti-inflammatory drugs. For these purposes two transgenic lines have been used: neutrophil-specific Tg (mpx:GFP)/114 and macrophage specific Tg (mpeg:mcherry). Different reference compounds with known anti-inflammatory effect were chosen and their doses selected after an MTC (Maximum Tolerated Concentration) assay carried out in 3 days post fertilization (dpf) embryos (when the innate immune system is already in place). To determine compounds toxicity at the innate immune system level, embryos were exposed to fresh dose for 48 h and the population of neutrophils and macrophages was quantified by fluorescence microscopy. Inflammation was induced by sterile injury of the tail fin and neutrophil recruitment to the wound site was assessed at 4, 6 and 12 h post injury in the presence of reference compounds using a partially automated platform. The ability of the compounds to suppress the expression of inflammatory genes (Il1b, Inf-a) was also evaluated by quantitative PCR. This zebrafish assay shows to be a cost-effective assay over mammalian models for the identification of new anti-inflammatory drugs as well as for the evaluation of immunotoxicity.
Neutropenia is a dose-limiting toxicity associated with immunomodulatory anticaner agents. One mechanism of drug-induced neutropenia is the inhibition of maturation of neutrophil blasts in bone marrow. We developed an in vitro assay to differentiate bone-marrow-derived CD34+ hematopoietic stem cells (HSCs) into mature neutrophils from cyonmolus monkeys for which in vitro neutrophil maturation has not been described. Commercially available CD34+ HSCs from cyonmolus monkeys were cultured in media supplemented with growth factors to induce expansion and myeloid commitment followed by neutrophil maturation. Examination of May-Grunwald Giemsa stained cells suggested that morphological changes were consistent with differentiation of CD34+ HSCs into mature neutrophils. The staining further revealed that mature neutrophils appeared in cyonmolus monkey CD34+ HSC cultures 5-6 days earlier than from cultures of human CD34+ HSCs under similar conditions. Neutrophil maturation was also evaluated by flow cytometry for cell surface markers: CD34 for early progenitor cells; CD33 for myeloblasts, promyelocytes, myelocytes, and metamyelocytes; and CD11b for metamyelocytes, band, and mature neutrophils. During the expansion and commitment phase, CD34 expression decreased and CD33 expression increased, consistent with the appearance of committed neutrophils. CD33 levels began decreasing and CD11b began increasing, consistent with the presence of mature and hyper-segmented neutrophils. These results demonstrate that CD34+ HSCs from cyonmolus monkeys differentiate into mature neutrophils in vitro. This assay will be useful for evaluating the potential of immunomodulatory compounds to induce neutropenia and for comparing nonclinical toxicity study findings to potential risk for humans.
Enzyme-linked immunosorbent assay (ELISA) is an extremely sensitive cell-based assay for detection of secreted effector molecules from immune cells. Here, we investigated the detection of IFNγ secreted from cynomolgus macaque peripheral blood mononuclear cells (PBMC) in response to cytomegalovirus (CMV) via the use of a UL-83 (pp65) peptide pool. Inherent variability in this highly sensitive assay means that acceptance criteria common to ELISA validation may not readily apply to all aspects of a validation effort for this type of assay. Additionally, significant variability in the execution of the assay can be introduced by small changes in input material processing. Further, the use of ELISpot assay in support of drug development requires bespoke validation per test article or antigen and therefore a robust validation study design to successfully optimize and execute in support of a standard toxicology study is needed. Here, we investigated critical parameters for successful ELISpot assay execution including: 1) optimal conditions and cell density for isolation, cryopreservation and thawing of PBMCs; 2) IFNγ responses from fresh and frozen PBMCs; 3) precision (intra-assay, inter-assay); 4) sensitivity and linearity; 5) antigen-specific spot count settings, detection, and sensitivity optimization for a given ELISpot reader system. We share lessons learned in our efforts to validate this assay and discuss the appropriate strategy to rigorously investigate the development of a test article, or antigen-, specific deployment of this assay in a GLP laboratory space.

Development of a Full-Thickness 3D Autologous Skin Equivalent Model to Determine Immunogenicity of Therapeutics


There is growing demand for human-based assays to test compounds/therapeutics for adverse immune reactions prior to entering clinical trials. Human skin equivalent models are useful in vitro testing platforms. We describe a unique full-thickness 3D autologous skin equivalent model made from primary human tissue which is representative of normal human skin and an autologous platform for testing therapeutics under development. Primary human fibroblasts and keratinocytes were grown from healthy volunteer tissue. The full-thickness skin equivalent model was generated by first forming a dermal equivalent by culturing fibroblasts on a scaffold before adding donor-matched keratinocytes and culturing at the air-liquid interface. Histology and immunofluorescence for protein markers of epidermal differentiation (involucrin and cytokeratin 14) and for dermal collagen (Picro Sirius red) was completed and compared to normal skin. We co-cultured the model with activated peripheral blood mononuclear cells (PBMCs) and observed similar immune damage to that in our skin explant assay. Immunofluorescence staining for heat shock protein 70 (HSP70) as a marker of apoptosis was used to confirm these findings. We also used the model to screen for adverse immune events to known positive and negative control therapeutic monoclonal antibodies, similar to our skin explant assay, but replacing the skin explant with the 3D autologous skin equivalent model. Our model was representative of normal human skin, showing similar structure observed by haematoxylin and eosin staining and positive immunofluorescence staining for protein markers of epidermal differentiation and skin structure (involucrin, cytokeratin 14 and Picro Sirius red). Our data also suggest this model could be used to detect adverse immune events to positive control therapeutic monoclonal antibodies with damage in the model as shown by positive HSP70 staining. No HSP70 staining was observed in the 3D skin model treated with negative control monoclonal antibody. We have generated an autologous 3D skin equivalent model, representative of normal human skin. Our data has shown this to be a useful platform for the detection of adverse immune events when testing biologics under development.
Dosage Scaling in Mouse Models for Binge Drinking: An Empirical Approach

S. B. Pruett, W. Tan, B. Nanduri, and G. Howell III. Mississippi State University, Mississippi State, MS.

Allometric dosage scaling between mouse and human is often done by expressing the dosage as dose per meter squared of body surface area rather than dose per body weight. This allometric conversion requires multiplying the dosage in humans by 12.3 on the basis of dose per body weight to obtain a biologically equivalent dosage in mice. However, this was derived from studies using one class of toxicants, chemotherapeutic agents for cancer. Subsequent work has shown that the conversion factor required to achieve biologically equivalent dosages varies considerably from one drug or chemical to another. Therefore, we sought to determine biologically equivalent dosages of ethanol in mice and humans using an empirical approach. We compared dosage (g/kg) to area under the blood ethanol concentration vs. time curve (AUC) for data from the literature for humans and from our own data (plus data from another lab for confirmation) for mice. We calculated the AUC using our original results that showed ethanol concentrations over time at various ethanol dosages. The results indicate that the linear relationship between dosage and AUC is similar for mice and humans (the lines are parallel). However, the x-intercepts are highly significantly different with 2.5-S fold higher dosages required in mice to achieve the same AUC as compared to humans. We also observed that both AUC and peak blood ethanol concentrations correlated significantly with induction of a corticosterone response in mice, indicating that AUC is a good indicator of biological effects. The results demonstrate that a dosage of 2 g/kg in humans produces similar AUC values as a dosage of 5 g/kg in mice, and a dosage of 1 g/kg in humans produced similar AUC values as 4 g/kg in mice. Thus, concerns in the ethanol research community about high ethanol dosages (5-6 g/kg) used in binge drinking experiments in mice seem to be unfounded. In fact, to match biological effects of ethanol in humans and mice, dosages must be greater for mice than for humans. This work was supported by R01 AA095505. S. Pruett is supported by P20 GM103646.

Immune Effects of Inorganic Arsenic Exposure on Mouse Bone Marrow-Derived Macrophages


In many parts of the world, including the United States, inorganic arsenic (iAs) contaminates groundwater used for drinking, food production, and irrigation. The World Health Organization set a 10 μg/L safety limit for arsenic in drinking water. Yet still as many as 140 million people worldwide may be exposed to drinking water with arsenic contamination at levels above that threshold. Elicits a broad range of adverse health effects, arsenic is a confirmed carcinogen by the IARC and also causes increased susceptibility to infectious diseases such as tuberculosis, indicating that arsenic affects the immune system. The purpose of this study is to elucidate the effects of arsenic on the developing immune system with an in vitro mouse bone marrow-derived macrophages exposure paradigm. Briefly, macrophages were cultured from larval rat bone marrow harvested from day 17 pups. The rats were dosed with different levels of iAs (0.0001 - 1 μM) either during or after macrophage differentiation, and stimulated with either LPS or PamCyS (Toll-like receptor 4 and 2 ligands, respectively). Culture supernatant was analyzed for cytokines and NO production. S2-plex cytokine analysis revealed differences between iAs-treated and untreated macrophages, with and without stimulation by LPS or PamCyS. For example, MCP-1, a cytokine that regulates chemotaxis of monocytes and basophils, is downregulated with increasing iAs doses applied after macrophage differentiation. Additionally, exposure to iAs alters macrophage nitric oxide (NO) production in a sex-dependent manner. Stimulated female samples yielded a U-shaped response with increased levels with by iAs, while male samples exhibited a negative linear trend. NO has been shown to produce both antimicrobial and antitumor activity. Therefore, for the host, disruption or alteration of macrophage NO production together with a suppressed proinflammatory cytokine response could lead to increased susceptibility to infectious agents and cancer. Further mechanistic investigation and pathway analysis may explain how arsenic specifically targets macrophages in a sex-dependent manner and alters downstream events, such as activation of immune cells essential for host protection. Research such as this contributes to our understanding of the full spectrum of adverse health effects of arsenic exposure and may help inform future policies on drinking water standards. Supported by NIHES R00ES024808 (FS) and T32ES07141 (EL KR).

Cigarette Smoke Extract May Induce Lysosomal Storage Disease-Like Adverse Health Effects

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Cigarette smoke is known to be associated with the incidence of a variety of pulmonary diseases, and alveolar macrophages are a key player in the defense mechanism against inhalable toxicants. Herein, we found that a hydrophilic fraction in smoke extracts from 3R4F reference cigarettes (CSE) contains high concentrations of volatile substances compared to cigarette smoke condensate (amphoretic fraction). We also identified the toxic mechanism of CSE using MH-S, a mouse alveolar macrophage cell line. CSE decreased cell viability accompanying increased LDH release. Additionally, mitochondrial volume was decreased, and a decrease of catalase activity and intracellular calcium concentration and decrease of ER and lysosome volume at the highest dose. More interestingly, damaged organelles accumulated in the cytosol, and CSE-containing particles specifically penetrated to mitochondria. Meanwhile, any significant change in autophagy-related protein expression was not found in CSE-treated cells. Following, we evaluated the effects of CSE on secretion of inflammatory-related cytokines and chemokines, considering the relationship between organelle damage and the disturbed immune response. Very importantly, we found that expression of innate and adaptive immunity-mediated mediators is disrupted following CSE exposure. Taken together, we suggest that CSE may cause the accumulation of damaged organelles in the cytoplasm by impairing selective autophagic function. In addition, this accumulation is responsible for the inadequate ability of immune cells to repair damage of lung tissue following exposure to CSE.

IL-4 Administration or Zinc Supplementation Mitigates Aggravated Thymus Atrophy in Zinc-Deficient Rats

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Nutritional zinc deficiency leads to immune dysfunction, including inflammatory diseases. We previously showed that inflammation induced by zinc deficiency can be inhibited by IL-4 administration or recovered by zinc supplementation. Furthermore, mitigation of thymus atrophy was observed by IL-4 administration or zinc supplementation. Here, we investigated whether the macrophage subtype is involved in mitigating thymus atrophy in zinc-deficient rats. Five-week-old male rats were fed a standard diet (17 g/day), and two groups were fed a zinc-deficient diet (n = 7 each). Each group was also injected with saline or IL-4 (zinc-deficient/IL-4 i.p.). Another group of rats were treated and untreated macrophages, with and without stimulation by LPS or PamCyS. For example, MCP-1, a cytokine that regulates chemotaxis of monocytes and basophils, is downregulated with increasing iAs doses applied after macrophage differentiation. Additionally, exposure to iAs alters macrophage nitric oxide (NO) production in a sex-dependent manner. Stimulated female samples yielded a U-shaped response with increased levels with by iAs, while male samples exhibited a negative linear trend. NO has been shown to produce both antimicrobial and antitumor activity. Therefore, for the host, disruption or alteration of macrophage NO production together with a suppressed proinflammatory cytokine response could lead to increased susceptibility to infectious agents and cancer. Further mechanistic investigation and pathway analysis may explain how arsenic specifically targets macrophages in a sex-dependent manner and alters downstream events, such as activation of immune cells essential for host protection. Research such as this contributes to our understanding of the full spectrum of adverse health effects of arsenic exposure and may help inform future policies on drinking water standards. Supported by NIHES R00ES024808 (FS) and T32ES07141 (EL KR).

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supplementation groups, thymus structure was recovered by intake of the standard diet. Therefore, there was no significant change in total macrophage and CD4+CD8+ double-positive cell numbers in the thymus.

### 2293 Effects of the Food Additive tBHQ on OVA-Elicited Food Allergy in Mice
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Immune-mediated adverse reactions to food allergens are rising at an alarming rate, globally, for reasons that are not completely understood. Although many factors such as microbiota, diet, obesity, and environmental chemical exposure have been proposed to contribute to this marked increase, the identification of specific causative factors has remained elusive. Previous data from our lab demonstrated that tert-butylhydroquinone (tBHQ), a food additive present in many processed foods, promotes polarization of T cells towards a Th2 lineage, a key step in the development of allergy. Here we tested the hypothesis that tBHQ, at concentrations relevant to human exposure, exacerbates the allergic response in ovalbumin (OVA) sensitized mice. Female Balb/c mice (4 weeks old) were provided control diet or diet with 0.001% tBHQ for the duration of these studies. Mice were exposed to OVA once per week for 4 weeks during the sensitization phase. Sensitization to OVA was assessed by the rise in OVA-specific IgE and IgG1. Upon oral challenge, mice were monitored for hypothermia shock response (HSR) and mast cell protease (mMCP-1) response. Although sensitization with OVA elicited a robust OVA-specific IgE antibody response in both the control and tBHQ diet groups, IgE levels were markedly higher in the mice on the tBHQ diet as compared to control diet. Likewise, in response to OVA challenge, a greater decrease in body temperature was observed in the mice on the tBHQ diet compared to control animals. Furthermore, the mMCP-1 response to OVA challenge was 3-fold greater in the mice on the tBHQ diet as compared to control diet. In addition, flow cytometry showed both mast cell population (CD117+ FcεRI+) and T helper 2 (Th2) cells (CD4+ GATA-3- IL-4+) population in splenocytes from the tBHQ-treated group were increased compared to control. Lastly, induction of IL-4, IL-5 and IL-13 was greater in splenocytes derived from mice on the tBHQ diet in an ex vivo recall response assay. Taken together, these data suggest that exposure to tBHQ through the diet promotes OVA sensitization and exacerbates anaphylactic response to OVA challenge in a mouse model of food allergy.

### 2294 In Situ Immunization with a TLR9-Agonist Enhances Survival in HNSCC
University of Iowa, Iowa City, IA.

Immunotherapy involving immune checkpoint inhibitors anti-PD1 and anti-CTLA4 antibody has shown promise for the treatment of head and neck squamous cell carcinoma (HNSCC). However, only a subset of HNSCC patients respond to these therapies. Therefore the identification of alternative immunotherapeutic strategies is necessary to improve HNSCC patient outcomes. CMP-001 is a novel toll-like receptor-9 (TLR9) agonist that consists of an unmethylated CpG motif-rich G10 oligonucleotide encapsulated in virus-like particles. In situ vaccination of CMP-001 is believed to activate tumor-associated plasmacytoid dendritic cells (pDCs) leading to tumor antigen presentation to T cells and anti-tumor T cell responses. This study is designed to investigate the therapeutic and abscopal effect of CMP-001. C57BL/6 mice were subcutaneously inoculated with murine mEERL HNSCC cells on both left and right flanks of each mouse. Three doses of CMP-001 was administered intratumorally into the left tumor. Tumor (injected and distant) growth, abscopal response, and survival were monitored. Results indicate that in situ vaccination of CMP-001 can significantly prolong mice survival, as well as suppress tumor growth on injected and distant sites over three weeks. The regression of CMP-001-injected tumors was durable. However, distant site tumors relapsed after the completion of treatment. In an ex vivo study we found an increase of CD4+ T cell infiltration at both injected and distant tumors in CMP-001-treated mice compared with controls. These results demonstrate the anti-tumor efficacy of CMP-001 and warrants further study of CMP-001 and other TLR9 agonists as a novel immunotherapeutic strategy for the treatment of HNSCC.

### 2295 E-cigarettes Adverse Effects and Nutrition Prevention
Southern University and A&M College, Baton Rouge, LA.

Chronic obstructive pulmonary disease (COPD) is one of the leading cause of death in United States. Cigarette smoking is one of the leading cause of COPD. Tobacco smoke contains more than 4500 chemicals, several of which are known carcinogens and/or can cause toxic effects leading to excessive inflammation and pathogenesis. With more than 7000 unique flavors and 450 brands commercially available, there has been recent surge in the sales of Electronic cigarettes (e-cigs), marketed as a safe alternative of conventional smoking. E-cigarettes are battery-operated devices which are used for heating e-liquids to produce vapors. Recent scientific reports present a controversial picture about this claim for e-cigs as safe alternatives. Studies now demonstrate that e-cigs can induce inflammatory responses almost similar to those by conventional cigarettes which may contribute to lung pathologies. On these lines, we investigated the effect of tobacco flavored e-cig vapor condensate (TF-ECVC; w/o nicotine) on immune related genes using human alveolar epithelial II cells (A549). Our findings revealed that AECII cells when exposed to the concentration of 2% or higher of TF-ECVC, show significantly reduced viability. We also observed TF-ECVC mediated increase in the production of cytokines and chemokines (CCL2, CXCL8, IL-1β and IL-6). Additionally, increased expression of the cystosolic receptor NOD-1; the membrane bound receptor TLR4; and Caveolin-1 (the protein associated with caveolae, a type of membrane raft) in TF-ECVC challenged cells provides further insights into signaling pathways regulated in A549 cells. Results obtained so far suggests for the possible regulatory role of lipid rafts (caveolae) in TL4/NOD1 signal-ing in TF-ECVC challenged A549. We also observed anti-inflammatory role of Urolithin A and Urolithin B in TF-ECVC challenged A549 cells in terms of cyto-kine/chemokine production and rescue of protein homeostatic mechanism. Further studies are in progress to determine detailed molecular mechanisms regulated by TF-ECVC and the role of Urolithins in mitigating the observed adverse effects.

### 2296 d-Limonene Modulates B Lymphocyte Activity and Viability
C. M. Lappas, and K. R. Patrick.
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Sponsor: C. Lappas, American Association of Immunologists

The cyclic terpene, d-limonene, which is a major component of several plant essential oils, is used widely as an additive in perfumes, soaps, foods and beverages, has been used clinically to dissolve cholesterol-containing gallstones, to relieve gastric acid-induced heartburn, and has been shown to possess chemopreventative and chemotherapeutic activity. Given the widespread use and clinical relevancy of the compound, it is surprising that only limited studies have investigated the effects of d-limonene on immune system function. It has been shown previously that d-limonene inhibits T cell activity and induces cell death at high concentrations. We show that d-limonene also modulates B lymphocyte activity and viability. B lymphocytes were purified from the spleens of C57BL/6 mice and activated by incubation with F(ab')2 goat anti-mouse IgM secondary antibody, IL-4 and anti-CD40 mAb for 24 hours at 37°C in 5% CO2. Cells were co-cultured with 0.5-8 mM d-limonene or vehicle control. Activation marker expression was measured by flow cytometry and cell viability was measured via trypan blue and annexin V and propidium iodide staining. The activation-induced expression of CD69 was inhibited by up to 72% as a result of exposure to d-limonene, the expression of CD68 was inhibited by up to 60%, while no significant effect on MHCII expression was observed. Treatment with 8 mM d-limonene induced B lymphocyte cell death, with lower doses having no significant effect on cell viability. These data indicate that d-limonene possesses immunosuppressant and cytotoxic activity. These immunomodulatory activities must be considered when evaluating therapeutic and commercial applications of the compound.

### 2297 Microcystin Exposure in Non-alcoholic Fatty Liver Disease Links Ectopic Intestinal Fibrotic Lesions: Role of Liver-Gut Crosstalk
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Intestinal fibrosis is a common complication of Inflammatory Bowel Disease (IBD) and in Crohn’s Disease globally, though it may remain subclinical without any intestinal obstruction. Studies also reveal that chronic inflammation such as non-alcoholic fatty liver disease (NAFLD) increases the risk of intes-
2299 High Dimension Biological Analysis Pipeline for Assessing the Immunotoxicity of Carbon Nanotubes

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The toxic effects of multi-walled carbon nanotubes (MWCNTs) are often associated with intracellular oxidative processes related to the generation of reactive oxygen species (ROS). In order to identify the effects of exposure to purified and unpurified MWCNTs on different biological targets, immunological, biochemical, and computational pathway analyses were carried out on samples from health human volunteers and in vitro models. MWCNTs with higher amount of metallic impurities caused a 1.8-fold increase in lipid hydroperoxide levels, while the number of mature T lymphocytes with reduced potential underwent more than a 3-fold increase. Whole genome transcriptomics in lung epithelium cells (A549) and keratinocytes (HaCaT) showed that gene expression is significantly modulated after exposure to purified and unpurified MWCNTs. 48-hour exposure resulted in approximately 500 genes expressed differentially in the case of MWCNTs with different levels of impurities. Similar results were found when we analyzed the data isolating the 25 genes involved in the molecular pathway associated to oxidative stress. The persistence of the induction of genes driving chemokine and cytokine-signaling-mediated inflammation showed the importance of the inflammatory effects of MWCNTs, and in particular the unpurified ones. Pathway analysis showed significant modulation of genes related to the NFκB pathway after exposure to unpurified MWCNTs, resulting from oxidative stress induction. This may serve as a parameter of the season’s circulating strains of virus. Despite improvement in capabilities to predict prevalent strains and rising yearly vaccinations, millions of Americans continue to suffer from influenza infection each year. Thus, it is important to consider the factors which may be decreasing the effectiveness of vaccines. We previously showed that a common food preservative, tert-butyldihydroquinone (tBHQ), impairs human CD4+ T cell activation and shifts murine CD4+ T cell polarization away from an antiviral phenotype. Accordingly, we hypothesized that consumption of tBHQ would impair the memory response to viral infection. To test this, we fed mice a diet containing 0.0014% tBHQ or a tBHQ-free diet. The mice were first infected with influenza x31 (H3N2), and 28 days later the mice were subjected to a heterosubtypic infection with influenza A/PR/8/34 (H1N1). After seven days, tissues were collected and effects of dietary tBHQ on immune cells were studied. tBHQ consumption resulted in increased expression of CD25 and CD69 in T cells, indicating greater cellular activation. Further, the effector function of T cells was heightened in the tBHQ group, characterized by increased CD107a and granzyme B expression. Likewise, tBHQ-exposed mice demonstrated lower levels of T cells which recognized influenza antigens, suggesting a robust immune response which lacked pathogen specificity, characteristic of a primary infection response as opposed to a memory response. The mice fed tBHQ also demonstrated higher mRNA expression of inhibitory proteins, CTLA-4 and IL-10, and more regulatory T cells known to suppress the immune response during influenza infection. Notably, this is the first study to show these effects. Lastly, after secondary infection the tBHQ-exposed mice experienced more severe weight loss and prolonged recovery compared to the control mice. Taken together, our data suggest that consumption of physiologically relevant doses of tBHQ results in impaired heterosubtypic immunity to influenza infection. This work was supported by NIH grants ES024966 and GM092715.
Nitro-oleic fatty acid (NOA) is formed by the nitration of the olefinic carbon to yield a potent electrophilic compound. NOA can modify cytostatic residues via Michael addition to alter protein function. It has been shown to reduce inflammatory cell activation within the cardiovascular system, but it has not been studied within the lung. Intratracheal bleomycin (ITB) administration is a well-established model of pulmonary inflammation and acute lung injury; therefore, in this study we examined whether intratracheal administration of NOA reduced bleomycin-mediated pulmonary inflammation. C57BL/6 male mice were administered bleomycin (3U/kg) or control (PBS) intratracheally. Half of the bleomycin or control animals received NOA (50μg) in the same volume as bleomycin 72 hours later. All animals were given in a total volume of 50μl. Bronchoalveolar lavage (BAL) and lung tissue were collected 7 days post ITB. As previously seen, mice treated with ITB lost a significant amount of body weight; however, addition of NOA mitigated this loss (-2.3±0.94 vs -4.0±1.83 g). ITB treatment increased lung leak as shown by increased BAL protein (67±22.2 vs 413±54.7 mg/mL), which was also reduced by NOA (50±35.1 vs 355±43.4 mg/mL). Histology revealed cell infiltration and tissue injury in ITB mice that was not reduced with the NOA. Flow cytometry of BAL cells demonstrated loss of Siglec F-+/F4/80-+/CD45-+/CD115-+ macrophages with ITB (95±3.3 vs 37±6.6%). Analysis of CD11b+/Gr1+ cells showed an increase in non-resident macrophages (4±5.8 vs 19±0.9%, p=0.1%) that was decreased by NOA (34±3.8%). To measure the effects of bleomycin and NOA on interstitial cells a lung digest was performed. Mesenchymal cells (CD31-, CD45-, Sca-1+) demonstrated an increase in CD44 and CD90 expression in response to ITB (3±2.0 vs 23±1.0%; 43±2.3 vs 74±2.6%) which was significantly reduced by NOA (19±0.9%; 70±2.3%). Single cell analysis of mesenchymal and perivascular endothelial cells (CD31+,-; CD45-,+) showed expression of the profibrotic protein ZEB1 was induced by ITB and that OANO significantly decreased ZEB1 by 3±0.94% vs 23±1.0%; 43±2.3 vs 74±2.6%) which was reduced by OANO (19±0.9%; 70±2.3%). To measure the effects of bleomycin and NOA within the lung lining at baseline. These findings suggest that treatment with NOA opposes ITB-mediated pro-inflammatory cellular activation and may do so by altering resident cell function and favoring resolution.

Manipulating the immune system to achieve therapeutic efficacy in cancer treatment has led to a new class of drugs that brought lasting remissions to many patients who had run out of options. The development of new drug candidates. Therefore, the goal of this study was to develop an NHP vaccination model that specifically elicits a CTL response, in order to evaluate the efficacy of the cytokotoxic T lymphocyte (CTL) response. To achieve this, MHC-genotyped Mauritian cynomolgus macaques (MCMs) were immunized with 3 replication incompetent recombinant adenovirus serotype 5 (Ad5) vectors, each containing the coding sequence for mesenchymal and perivascular endothelial cells (CD31+,-; CD45-,+) showed expression of the profibrotic protein ZEB1 was induced by ITB and that OANO significantly decreased ZEB1 by 3±0.94% vs 23±1.0%; 43±2.3 vs 74±2.6%) which was reduced by OANO (19±0.9%; 70±2.3%). To measure the effects of bleomycin and NOA within the lung lining at baseline. These findings suggest that treatment with NOA opposes ITB-mediated pro-inflammatory cellular activation and may do so by altering resident cell function and favoring resolution.

During an innate immune event B-cells are activated to produce immunoglobulins (Ig). Functional Ig production is partially controlled by the immunoglobulin heavy chain locus (IGH) which contains two 3'regulatory regions (3'IGHR). Three enhancers (hs3, h1, h2, hs4) are contained within each 3'IGHR and are thought to influence transcription, Ig class switch recombination (CSR) events. During CSR B-cells switch into different Ig isotypes (IgG1, IgA1-2, IgM, IgE). The human hs1 enhancer within the 3'IGHR is polymorphic in that it contains a 53bp invariant sequence (IS) that can be repeated one to four times in tandem. Multiple autoimmune disorders have been associated with the hs1.2 polymorphism as well as a sensitivity to xenogenous chemicals, such as 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'IGHR are correlated with inhibition of secreted Ig. However, TCDD has been shown to activate the human hs1 enhancer and produce differential effects on Ig secretion, i.e. decreased IgG but increased IgE. These results highlight the potential species differences in the hs1.2 activity and 3'IGHR 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. Utilizing CRISPR/Cas9 gene editing within a human B-cell line (CL-01) that can be induced to express the different Ig isotypes, we targeted the polymorphic IS in the hs1.2 enhancers to induce a deletion in the number of IS repeats. We identified several clones with an altered hs1.2 genotype that corresponded to changes in basal and stimulated IgM and IgG secretion, as well as changes in the mRNA expression of the different Ig isotypes. To measure the effects of bleomycin and NOA within the lung lining at baseline. These findings suggest that treatment with NOA opposes ITB-mediated pro-inflammatory cellular activation and may do so by altering resident cell function and favoring resolution.

Type 1 diabetes (T1D) is an autoimmune disease characterized by T-cell mediated destruction of insulin-producing pancreatic beta cells. Both genetic and environmental factors have been shown to contribute to T1D development. One candidate environmental factor implicated in T1D susceptibility is the diet. A potential connection between the diet and regulation of immune function is the aryl hydrocarbon receptor (AhR), a ligand activated transcription factor that modulates the expression of metabolizing enzymes (e.g. Cyp1a1) as well as immune genes. Previous studies with the high affinity synthetic AhR ligand, 11-CBQ, demonstrated that strong AhR activation leads to reduced severity of insulin. The working hypothesis for this study is that AhR activation by the dietary ligand, indole-3-carbinol (I3C) will suppress development of T1D. I3C is found in cruciferous vegetables such as broccoli, kale, and Brussels sprouts. Mice were fed a synthetic plant-free diet (AIN93M) supplemented with 2000ppm I3C. Based on the average measured food consumption of 2.5 g/day, the dose of I3C was equivalent to 250 mg/kg/day. Initially, a 1-week pilot study was conducted to determine the extent by which dietary I3C activates AhR. AhR was highly activated in the small intestine as measured by Cyp1a1 induction (5475±4205-fold increase in the duodenum, 650±269-fold in the jejunum, and 26810±25939-fold in the ileum). Cyp1a1 was also induced systemically, although to a much lower extent (10.1±3.2-fold in the liver, and 2.8±0.5-fold in the PLN). In a follow-up study, mice were fed an I3C supplemented diet starting at 7 weeks of age and insulitis was scored at 12 weeks of age. Surprisingly, dietary I3C significantly increased insulitis, which diverges from our findings with 11-CIBQ. One explanation for this finding could be that 2000ppm I3C suboptimally activated AhR. In our studies with 11-CIBQ (45 mg/kg), AhR was activated systemically resulting in hepatic Cyp1a1 induction three orders of magnitude higher than in mice treated with I3C. Our finding that inadequate AhR activation worsened insulitis is consistent with our previous findings that low levels of AhR activation promotes hyperinflammatory responses, whereas high levels of of mesenchymal and perivascular endothelial cells (CD31+,-; CD45-,+) showed expression of the profibrotic protein ZEB1 was induced by ITB and that OANO significantly decreased ZEB1 by 3±0.94% vs 23±1.0%; 43±2.3 vs 74±2.6%) which was reduced by OANO (19±0.9%; 70±2.3%). To measure the effects of bleomycin and NOA within the lung lining at baseline. These findings suggest that treatment with NOA opposes ITB-mediated pro-inflammatory cellular activation and may do so by altering resident cell function and favoring resolution.
AhR activation suppresses the immune response. In future studies, we will use AhR knockout mice on the NOD background to demonstrate that I3C acts through AhR to alter insults severity.

**2307** Breaking Tolerance: A Case of Immune-Mediated Thrombocytopenia after Administration of BMS-986156 An Anti-GITR Antibody to Cynomolgus Monkey


Glucocorticoid-induced TNFR-related protein (GITR), is a co-stimulatory immune receptor expressed on activated T cells, with highest expression observed on regulatory T cells. BMS-986156, an agonistic IgG1 monoclonal antibody specific for GITR, enhances T effector and regulatory functional markers in vivo via activation of NFKB and increased cell survival and by depleting regulatory T cells via antibody-dependent cellular cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP). In a 3-month intermittent intravenous (QW) toxicity study with a 10 week post-dose recovery, cynomolgus monkeys (up to 6 sex/group) were administered 0, 10, 40, or 150 mg/kg BMS-986156. One female monkey administered 150 mg/kg BMS-986156 (AUC [0 - 168] 593,000 µg • h / mL, approximately 75× the steady state exposure at clinical dose of 240 mg Q2W, well above the clinically administered dose) had substantial decreases in platelets at the end of dose and during the recovery period. One monkey administered 150 mg/kg BMS-986156 was negative for a potential immune-mediated cause. Serum from the female monkey with thrombocytopenia had increased platelet-specific antibodies relative to concurrent vehicle control treated female monkeys and relative to other female monkeys administered 150 mg/kg BMS-986156 (up to 23×). The presence of platelet-specific antibodies in the serum of this female monkey provides a potential mechanism of the observed thrombocytopenia. Given the agonistic potential of anti-GITR immunotherapy in enhancing immune responses, the presence of autoantibodies to endogenous platelet surface proteins was considered likely due to pharmacology.

**2308** Neuroimmunotoxicological Perspectives of Gulf War Illness

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Gulf War Illness is a systemic, multi-symptom condition affecting veterans of the Gulf War who have been exposed to chemical warfare agents such as rockets filled with sarin and cyclosarin, both of which are nerve agents that are extremely toxic. Symptoms can include neurological dysfunction, mental illnesses such as PTSD, depression and anxiety, gastrointestinal bleeding and severe upset, as well as disorders affecting other systems in the body, but the immune system, in particular, is placed under extreme stress by these neurotoxins due to the cascades activated within the complement component of the immune system; this complement component serves as a crucial piece in host defense, providing innate and acquired immunity for the body. When it is affected by these neurotoxins, the inflammatory response is activated and this increases the likelihood for conditions like Parkinson’s, AMD and Alzheimer’s Disease, as well as a plethora of other conditions that are unexplainable by the patient’s family medical history. Our previous studies have indicated a higher level of autoantibodies, compared to neuronal specific proteins. The consistent presence of such autoantibodies in the systemic circulation can result in activation of other immunological factors. The main objective of this study is to compare the complement component of the healthy and ill GW veterans. Although complement plays a major role in host defense, its role in inflammatory responses is alarming. During normal healthy conditions, the complement is regulated by membrane-associated and soluble cytotoxic proteins, and when dysregulated, it induces damage to the host cells leading to pathological conditions initiating an autoimmune response. The activation of complement cascade is by the classical, alternative, or lectin pathways. The alternative pathway is continuously activated in vivo through spontaneous C3 thioester bond hydrolysis, resulting in the formation of C3 convertase, generating C3b, which in turn activates several proteins. Factor H is one of the main regulators of the activation of the alternative pathway, which promotes dissociation by preventing the formation of the C3 convertase enzyme. Our preliminary results on complement component C3 by ELISA showed an increased level in GW veterans compared to healthy GW veterans (p-value less than 0.001) and our forthcoming results will indicate the comparative levels of other complement components and C reactive protein to that of the autoantibodies. Supported by DOD W81XWH-18-1-0454.

**2309** Development and Validation of Assays for Detection of Anti-HPV Antibodies and Neutralization of HPV Viruses

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Immunoassays were developed for detection of anti-HPV antibodies and HPV virus neutralization in rabbit serum after IM administration of HPV16L1-16GSI synthetic peptide vaccine (Kinzbaier et al., 1992; Roden and Wu, 2006). The validated mouse ELISA assay was then modiﬁed to measure the L1 and L2 (17-35) Abs titers simultaneously in a single serum sample (5 µL) using a multiplex assay. The standard curves for L1 and L2 were linear (delta > 30%) for at least seven 2-fold consecutive dilutions from 12,800X to 819,200X. All curves provided excellent inter-plate precision (CVs from 2.16% to 11.98% for L1 and 5.61% to 9.19% for L2). Following testing of 10 blank matrix samples from different sources, the maximum matrix interference was seen at the level of 0.0078 EU/ml for L1 and 0.0231 EU/ml for L2. The high level quality controls (1 EU/ml) for L1 and L2 at 12,800X dilution and 25,600X dilution showed very good intra-plate and inter-plate precision (L1 - CVs from 2.0% to 12% for intra-plate and 16.4% to 19.2% for inter-plate assay; L2 - CVs from 1.2% to 10.2% for intra-plate and 11.9% to 16.0% for inter-plate assay). The screening cut points were established at 12,800X dilution for signals at 192 and 223 for L1 and L2, respectively. The neutralization assay in rabbit and human plasma was developed to detect neutralizing antibodies. Several batches of furin-cleaved HPV18-Luc were prepared and analyzed by gel electrophoresis and neutralization assays. Plasmid plasmid and pLucfLuc plasmid were co-transfected into 293T cells and the cells were collected after 48 hours. The cells were subjected to lysis buffer and incubated for 48 hours at 37°C for virus maturation. The lysate was collected and subjected to Opti-prep density-gradient ultracentrifugation. More batches of furin-cleaved HPV18-Luc were prepared and viral activity and neutralization studies with rabbit sera were performed using different batches of the psuedovirus as well as from the combined stock of fcPsV18 from all the batches. The neutralization assay will be validated using the combined stock and will be used for future GLP rabbit studies with the HPV vaccine. This assay showed high titers of HPV antibodies induction and sufficient virus neutralization by Day 45 in two pilot rabbit studies. This work was funded by National Cancer Institute, contract number: HHSN261201500261.

**2310** In Vitro Skin Tests for the Detection of Sensitization, Immunotoxicity, and Assessment of Relative Potency


Sensitization to chemicals and cosmetics resulting in allergy is an important health issue. Until recently, the most favorable method to test compounds for sensitization was the mouse local lymph node assay (LLNA). Here we describe a human in vitro skin explant test for identification of sensitization hazards and the assessment of relative skin sensitizing potency. This method uses a human autologous system to test for sensitivity and adverse reactions to compounds, in which activity is measured as histopathological grading of skin damage, caused by induced immune sensitization response, which correlates with T cell proliferation and IFN-γ production. Using this approach we have measured responses to 44 chemicals including skin sensitizers, pre/pro-haptens, respiratory sensitizers, non-sensitizing chemicals (including skin-irritants) and previously LLNA misclassified compounds (e.g. Nickel Sulphate). The skin explant test gave 95% specificity, 95% sensitivity, 95% concordance with the LLNA and a correlation coefficient of r=0.9 (p<0.0001). Additionally, it has proven to be a sensitive method for predicting allergy responses to cosmetics and can be used to determine adverse effects of repeat dosing. The assay was modified to aid preclinical prediction of immunotoxicity of therapeutic drugs such as monoclonal antibodies or small molecule drugs. We tested 17 commercial biologics in the skin explant assay (n=10) and correlated the results with clinical occurrence of adverse reactions. Results showed a statistically significant positive correlation between adverse clinical occurrences and a positive response in the skin explant test (r=0.815, p<0.0001). An analogue of the TGN1412 drug, which caused the Northwick Park trial disaster in 2006, was tested and gave an extreme positive response.
Role of Lymphocyte-Specific Protein Tyrosine Kinase (LCK) in Suppression of the Immunoglobulin M (IgM) Response in Human CDS$^+$ Innate-Like B Cells (ILBs)

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LCK is a well-characterized kinase involved in T cell receptor signaling but the role of LCK in human B cells is poorly understood. Recent studies have demonstrated that AhR-mediated suppression of the IgM response in human B cells involves upregulation of LCK. Interestingly, human ILBs, a heterogeneous population within the total B cell pool, express high levels of LCK. ILBs also express a high basal level of program cell death protein-1 (PD-1), an immune checkpoint inhibitor, which can suppress the IgM response, and is especially sensitive to stimulation by interferon gamma (IFNγ). Therefore, studies were conducted to determine the sensitivity of ILBs to AhR-mediated suppression of the IgM response and determine whether stimulation of ILB with IFNγ alters the levels of LCK, PD-1, and suppression of the IgM response by AhR activation. From these studies, AhR activation significantly upregulated total LCK and PD-1 proteins in ILBs, which correlated with significant suppression of IgM. In addition, IFNγ treatment significantly reduced the total LCK protein levels and increased AhR-mediated IgM suppression in ILBs. Collectively, results from these studies support the role of LCK in AhR-mediated suppression of IgM responses in human CDS$^+$ ILBs. In addition, ILBs are particularly sensitive to the effects of TCDD, likely due to their high expression of LCK. Supported in part by NIH ES002520 and ES004911.

Sex-Specific Effects of Chronic Arsenic Exposure on Influenza Pathology in Adult C57BL/6 Mice

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Inorganic arsenic (iAs) is a common drinking water contaminant, known immunotoxicant and is associated with increased risk of respiratory infection. About 200 million people globally are exposed to iAs in their drinking water at levels exceeding the World Health Organization’s safety standard of 10 ppb. Respiratory infections like influenza A virus (IAV) remain among the top causes of death worldwide. While a few previous studies demonstrate increased IAV susceptibility in iAs-exposed mice, none to date have assessed the role sex and iAs dose play in immunotoxicity and IAV pathogenesis. The aim of this study is to determine those sex- and dose-dependent immunotoxic mechanisms of iAs exposure on IAV disease susceptibility and pathogenesis in a mouse model. We hypothesize that exposure to iAs skews the immune response in a dose- and sex-specific manner, resulting in increased pathology and mortality in males and females. Exposed seven-week-old C57BL/6 male and female mice to iAs (sodium meta-arsenate) at 0, 10, 100 or 1000 ppb chronically via ad libitum drinking water. At 5 wk-of exposure, mice were intranasally infected with 10$^5$ 50% tissue culture infective dose (TCID$_{50}$) of mouse-adapted A/California/4/2009 (H1N1) (ma2009). Results demonstrate that exposure to all doses of iAs in female mice and 100 and 1000 ppb in male mice significantly increases clinical signs of disease pathology (ANOVA, p=0.0025) and mortality (log-rank survival analysis, p=0.0033) at 12 days post-challenge (DPC). Analysis of bronchoalveolar lavage fluid at 3 DPC reveals that IL-2 expression is reduced in males and females exposed to iAs, while many proinflammatory cytokines and chemokines were significantly increased at all doses in females and in males exposed to 1000 ppb iAs compared to control. We are currently investigating the source of the exacerbated innate inflammatory response and how iAs exposure leads to higher mortality in females compared to males. We are assessing lung viral titers and histopathology, and performing flow cytometric immune cell profiling in the lung and mediastinal lymph nodes following infection. Our data suggest the sex- and dose-dependent immunomodulatory effects of iAs in drinking water on influenza infection response and pathology. These results help shed light on the increased respiratory pathology seen in epidemiological studies of individuals exposed to high levels of drinking water iAs. Funding: ST32HL070534-35.

Hunting for Goldilocks: Trying to Identify a “Just Right” Dose for an OX40-Activating Antibody in Cynomolgus Toxicology Studies

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BMS-986178 is a fully human immunoglobulin G1 agonist monoclonal antibody that binds to human OX40, a co-stimulatory receptor expressed primarily on activated effector T cells and regulatory T cells. In preclinical studies, OX40 agonism has been shown to increase cytotoxic T-cell activity and enhance the antitumor immune response. Administration of a BMS-986178 mouse monoclonal antibody in mice with CT26 tumors demonstrated an inverted U-shaped curve, with increased antitumor activity at doses of ≤3 mg/kg but decreased antitumor activity at 10 mg/kg. Similarly, in vitro data with human T cells showed increasing T-cell activation as BMS-986178 increased to a certain concentration, after which T-cell activation decreased. Toxicology studies of BMS-986178 performed in cynomolgus monkeys were consistent with an inverted-U-shaped dose-response curve relating to immunostimulation. High doses of BMS-986178 (≥30 mg/kg intravenously weekly for 1 month) in cynomolgus monkeys showed immune suppression instead of stimulation as assessed by T-cell-dependent antibody and ex vivo recall responses to keyhole limpet hemocyanin (KLH), suggesting that high doses are in the immune-suppressing range of the inverted U-shaped curve. To identify a dose of BMS-986178 that can elicit the intended immune-stimulating pharmacological effects in cynomolgus monkeys, a second 1-month pharmacodynamics and toxicology study was conducted with weekly doses of BMS-986178 from 0.1 to 10 mg/kg to total of 5 doses (day 1 AUC$_{0-168}$ = 0.1 to 20× the 80-mq Q2W clinical AUC). BMS-986178-clearing antidrug antibodies (ADAs) formed after the second dose at ≤1 mg/kg; at 1 mg/kg, ADAs were associated with hypersensitivity reactions (transient tremors, redness, and/or swelling after the third and/or fourth dose). Furthermore, suppression of the T-cell-dependent antibody and ex vivo recall response to KLH were observed at ≥1 mg/kg. Therefore, these data indicate that there is no BMS-986178 dose in cynomolgus monkeys that allows for both the intended pharmacology and sustained exposure for ≥1 month and suggest that the cynomolgus monkey is not an optimal model for safety evaluation of BMS-986178.

Nickel Nanoparticles Enhance LPS-Induced Pro-Inflammatory Cytokine Production via a NF-κB-Dependent Pathway in Human Lung Epithelial Cells In Vitro and Promote Acute Lung Inflammation in Mice In Vivo

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Nickel nanoparticles (NINPs) are widely used in various industrial applications such as catalysts for the production of multiwalled carbon nanotubes. Thus, the toxicity of NINPs after inhalation exposure is a major concern for occupational lung diseases. Pre-existing respiratory disease or the sex of the individual, may influence one’s susceptibility toward exposure to NINPs. Pre-existing lung inflammation can be induced by lipopolysaccharide (LPS), a ubiquitous component of gram-negative bacteria. Both LPS and NINPs activate the toll-like receptor, TLR4. We hypothesized that NINPs would exacerbate LPS-induced lung inflammation through amplification of TLR4 signaling intermediates (e.g., NF-κB, MyD88) to enhance cytokine production and lung inflammation. In vitro: an immortalized human bronchial epithelial cell line (BEAS-2B) was stimulated with LPS, NINPs, or both. Cell supernatants and mRNAs were collected after 24 and 48hrs after the treatment to measure IL-6 and IL-8 mRNA and secreted protein by RT-PCR and ELISA, respectively. The lcb kinase inhibitor, 2-(aminobenzotriamino)-5- (4-fluorophenyl)-3-thiophencarboxamide (TPCA-1), was used to inhibit activation of NF-κB. To determine whether NF-κB plays a role in NINP exacerbation of LPS-induced inflammation, BEAS-2B cells were treated with TPCA-1 (5μM or 10μM) 30 minutes prior to LPS and NINP stimulation. In vivo: 8-week old male and female mice were treated by intranasal instillation with vehicle (0.1% Pluronic in PBS), LPS (Sug/kg), NINPs (4mg/kg), or both LPS and NINPs. Necropsy was performed 24hrs post-exposure. NINPs and LPS synergistically increased IL-6 and IL-8 production both at the mRNA and protein level in BEAS-2B cells. TPCA-1 blocked the synergistic increase of IL-6 and IL-8 caused by LPS and NINP co-exposure in BEAS-2B cells. The in vivo study also revealed that male mice were more susceptible to acute lung inflammation caused by either LPS and NINPs compared to female mice. Both LPS and NINPs co-exposure to NINPs and LPS would result in more severe lung inflammation in humans compared to either agent alone, especially in males. Funding: Supported by NIHES grant R01-ES020897, NIEHS Training Grant T32ES007046, and NSF Grant T5-022.
Industrialized countries are facing a significant increase in immune-mediated disorders, including allergy, autoimmunity and cancer, for which exposure to environmental factors, includingendocrine disrupting chemicals, may provide plausible explanation. RACK1 is a scaffolding protein involved in several signaling pathways, controlling essential cellular processes and important biological events, including immune response, and cancer. Our published data supports the existence of a complex hormonal balance, between glucocorticoids and androgens, in the control of RACK1 expression and immune cells activation, suggesting that RACK1 can be targeted by endocrine active compounds. This study aimed to investigate the regulation of RACK1 expression following exposure to selected estrogen active compounds, namely 17β-estradiol, diethylstilbestrol (DES), bisphenol A (BPA) and zearalenone, to define whether and to what extent they can modulate the transcriptional regulation of RACK1, and its implication in the modulation of innate immune responses. The human promyelocytic cell line THP-1 was used for all experiments. Cells were treated with increasing concentrations (highest concentration tested CV80) of the selected compounds for 6 and 16 h for the RACK1 promoter activity in transiently transfected with Δ1 luciferase reporter construct of the human RACK1 gene, 16 h for the mRNA expression and for 24 h for the protein expression. For functional testing, after 24 h of incubation with the selected compounds LPS (10 ng/ml) was added, and pro-inflammatory cytokine release assessed 24 h later by ELISA. All compounds modulated RACK1 expression and the response to LPS, confirming our working hypothesis. However, the results showed a different behavior of the analyzed substances. While 17β-estradiol and DES increased at low doses (1-2 nM) and reduced at higher doses (> 1 μM), BPA and zearalenone showed an increase at higher concentrations (> 1 μM) in the expression of RACK1, suggesting that different mechanisms are in place, not directly attributable to the estrogenic activity.

### 2316 Applying the Appropriate Assay Format for Cytokine Release Assays to Deliver the Appropriate Interpretation of Safety Risks

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Following the serious adverse events that resulted in severe cytokine storm responses in individuals dosed with TGN1412, it has been a health authority regulatory expectation that any compound that has the potential to modulate immune activation status is assessed with in vitro assays to predict the potential for activation of a cytokine release mechanisms. Over the last 10 years many investigators have assessed a variety of in vitro cellular assays for evaluation of this response but these do not always reflect the true biologic mechanisms taking place. This poster will review data generated for liquid phase, solid phase, and co-culture assay formats with positive controls (Anti-CD3 Monoclonal antibody, Anti-CD28 Monoclonal antibody, Anti-Her2 Monoclonal antibody and CD19/CD3 bi-specific T-cell engager) which are known to have different modes of action for induction of cytokine release. This data will be used to highlight the importance of choosing an appropriate assay format for characterisation of cytokine release. It will discuss how target biology considerations and test article structural considerations need to be considered for choosing the appropriate cytokine release assay format. Details of how the Fc region of a monoclonal antibody will govern the choice for a solid phase format and how co-culture systems with PBMCs and endothelial cells can be used for a greater physiological understanding will be presented. Utilising these data the poster will show how applying the appropriate assay format can be used to accurately predict the potential for cytokine storm for novel biologics.

### 2317 Epicutaneous Sensitization with Protein Allergens Differentiates Naïve T Cells into Not Only Th2 but Also Th17 Cells, Which Differs from the Sensitization with Chemical Allergens


It is well known that the sensitization with chemical allergens differentiates naïve T cells into TH1 cells in draining lymph nodes, which elicits delayed-type hypersensitivity reactions. IFN-gamma secreted by Th1 cells plays an important role in this process. Conversely, what types of effector T cells are differentiated in draining lymph nodes of mice sensitized by protein allergens such as enzyme is not well known. Furthermore, the types of hypersensitivity reactions elicited by protein allergens remain unclear. To address these issues, we investigated mice sensitized by papain enzyme as a protein allergen. Mice were sensitized by 24hrs exposure to papain twice a week for one month. The draining lymph node cells were then isolated and re-stimulated by papain. ELISA and FACS analyses of the cytokines synthesized in lymph node cells revealed that CD4+ IL-4+ Th(2) and CD4+ IFN-γ+ T(17) cells were differenti-ated in the draining lymph nodes of the mice sensitized by papain. However, CD4+ IFN-gamma+ T cells (Th1) were not detected. The sensitized mice were elicited by applying papain onto the ear to address the role of Th2 and Th17 cells differentiated in the papain-sensitized mice, and the allergic reactions in the ear were examined. Real-time PCR analyses revealed that IL-17 mRNA of Th17 marker was up-regulated in a time-dependent manner; however, IL-4 mRNA of Th2 maker was not detected at any time points. These phenomena were confirmed by immunofluorescent microscopy using a specific antibody for IL-17, suggesting that Th17, but not Th2, migrated into the ear after the elicitation. Additionally, the papain-sensitized mice possessed papain-specific IgE, and mast cell degranulation was observed after the elicitation. These findings reveal for the first time that the sensitization of papain enzyme differentiates naïve T cells into not only Th2 but also Th17 cells as effector T cells contributing to the allergic reactions. The generated Th2 produces IL-4 and is involved in IgE syntheses in the draining lymph nodes. Conversely, the generated Th17 cells migrate to the skin and produce IL-17 after elicitation.

### 2318 Effect of TCDD on B Cell Responses in the Spinal Cord in EAE

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Multiple Sclerosis (MS) is an autoimmune demyelinating degenerative disease of the central nervous system. A mouse model, experimental autoimmune encephalomyelitis (EAE), is most commonly used to study the pathogenicity of MS, which can be induced after administration of the self-peptide myelin oligodendrocyte glycoprotein (MOG35-55). The severity of EAE can be influenced by Th2 and Th17 cytokines. In our previous studies, TCDD upregulated FasL on B cells, especially the follicular B cells in the spleen and lymph nodes of EAE mice at day 18, suggesting that part of the mechanism for suppression of EAE is induction of a FasL-expressing regulatory B cell to induce apoptosis. The mechanisms by which TCDD upregulates FasL on B cells, especially the follicular B cells in the spleen and lymph nodes of EAE mice at day 18, are not well understood. To investigate the mechanism of TCDD on B cell responses in the spinal cord, we performed ELISA and FACS analyses of the cytokines synthesized in lymph node cells. We found that the number of infiltrating cells as well as CD4+ IFN-gamma+ T cells (Th1) were not detected. The sensitized mice were elicited by applying papain onto the ear to address the role of Th2 and Th17 cells differentiated in the papain-sensitized mice, and the allergic reactions in the ear were examined. Real-time PCR analyses revealed that IL-17 mRNA of Th17 marker was up-regulated in a time-dependent manner; however, IL-4 mRNA of Th2 maker was not detected at any time points. These phenomena were confirmed by immunofluorescent microscopy using a specific antibody for IL-17, suggesting that Th17, but not Th2, migrated into the ear after the elicitation. Additionally, the papain-sensitized mice possessed papain-specific IgE, and mast cell degranulation was observed after the elicitation. These findings reveal for the first time that the sensitization of papain enzyme differentiates naïve T cells into not only Th2 but also Th17 cells as effector T cells contributing to the allergic reactions. The generated Th2 produces IL-4 and is involved in IgE syntheses in the draining lymph nodes. Conversely, the generated Th17 cells migrate to the skin and produce IL-17 after elicitation.
2319 Low-Level Arsenic Exposure Impairs the In Vitro Differentiation of Mouse Bone Marrow Erythroid Progenitor Cells

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Anemia is a hematological disorder that affects millions of people worldwide. The prevalence of anemia is disproportionately high in arsenic endemic regions. Despite strong epidemiological evidence for the association between arsenic exposure and anemia, a clear mechanistic understanding of how arsenic contributes to anemia remains unknown. The goal of this study was to evaluate the effects of arsenite (As³⁺) and the As³⁺ metabolite, monomethylarsonious acid (MMA³⁻) on the development of bone marrow erythroid progenitor cells. To determine the effects of As³⁺ and MMA³⁻ on erythroid progenitor cell differentiation, we developed an in vitro model of erythropoiesis using primary mouse bone marrow hematopoietic progenitor cells (HPC). The progression of HPC through the stages of erythropoiesis was assessed based on cell surface marker phenotype using flow cytometry. The percentage of megakaryocyte-erythroid progenitors and colony-forming-unit-erythroid cells was significantly reduced after 48 h exposure to 100 and 500 nM As³⁺. MMA³⁻ significantly suppressed all erythro-megakaryocytic progenitor subsets. Evaluation of later-stages of erythroid differentiation revealed a significant suppression of erythroblast subsets at 24 h that persisted through 72 h with the 500 nM As³⁺ and 100 and 500 nM MMA³⁻ exposures. Throughout the differentiation time course, a significant increase in the percentage of early myeloid progenitor cells as well as more mature myeloid cells was observed with both As³⁺ and MMA³⁻. Our results show that As³⁺ and MMA³⁻ suppress the differentiation of erythroid progenitor cells starting at very early stages of erythropoiesis, possibly by skewing HPC lineage commitment in favor of myelopoiesis. Future studies will focus on characterizing mechanisms responsible for arsenic-induced hematopoietic imbalance and impairment of erythropoiesis.

2320 Prolonged Exposure to Sodium Arsenite Initiates Epithelial-Mesenchymal Transition in Human Renal Proximal Tubule Epithelial Cells (RPTEC/HTERT)

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Renal fibrosis develops in the glomerulus and tubulointerstitium as a result of cellular injury. The progressive decline in renal function that occurs in kidney disease is correlated with the degree of tubolointerstitial fibrosis. These fibrotic lesions are the result of myofibroblast activation, and subsequent production of collagen matrix, however the origin of myofibroblasts in the tubular epithelia remains controversial. In this study, we evaluated the potential of renal proximal tubular epithelial cells (RPTEC/HTERT) to transition into myofibroblasts via epithelial-mesenchymal transition by subjecting cultures to prolonged sodium arsenite exposure. Here we report that RPTEC/HTERT chronically exposed to moderate concentrations of sodium arsenite (4.5 micromolar) have lost the ability to perform apical-basolateral transport, and display morphological features consistent with a transition from tubular epithelial cells to myofibroblasts including elongated spindle shape, cytoplasmic projections, and decreased intercellular contacts. Confocal microscopy revealed the acquisition of several mesenchymal characteristics, including the upregulation of N-cadherin, vimentin, and alpha-smooth muscle actin with a concomitant decrease in E-cadherin and Ksp-cadherin. Further, these cells have substantially decreased E-cadherin and beta-catenin localized to the membrane, indicating the dissolution of adherens junctions. Together, these results provide evidence supporting the capability of proximal tubule epithelial cell de-differentiation in to myofibroblasts, consistent with pathological type II EBPs that prolonged arsenite exposure can initiate this process in proximal tubule epithelial cells.

2321 Enrichment of Genes Associated with Squamous Differentiation in Cancer-Initiating Cells Isolated from Arsenite Transformed UROtsa Cell Lines


Urothelial cell carcinomas (UC) with basal characteristics particularly squamous differentiation are generally classified as being more aggressive with poor outcomes. Our laboratory has shown that the immortal urothelial cell line, UROtsa when transformed with the environmental carcinogen arsenite gives rise to tumors exhibiting prominent focal areas of squamous differentiation. Cancer initiating cells (CICs) isolated from these transformed cell lines also form tumors in immune compromised mice with a histology distinguishable from the originating cell lines, including focal areas of squamous differentiation. These observations suggest that the CICs may represent the cell population that is originally transformed by arsenite exposure. The goal of this study was to determine the degree of difference in the gene signature pattern between the arsenite-transformed cell line and the CICs isolated from the cell line and determine if the CICs gene signature was enhanced for the genes related to squamous differentiation or other pathways involved in the development of UC. Our results demonstrate that differential expression of genes between the arsenite-transformed cell line and isolated CICs totals 4,415 transcript clusters and 3,469 identifiable genes. In addition, the CICs were highly enriched for genes involved in squamous differentiation, which include genes for keratinization and formation of the cornified, envelop. These genes (AZM1, CERS, DSG1, SPRR3, SPRR2E, SPRR4, SPRR2D, SPRR1B, SPRR2A, SPRR2B, SULT2B1, KRT1, KRT10, KRT16, KRTDAP, SCEI, ZFP750, KRT14, KRT6a, and KRT6b) have a role in structure, function or development of the stratified squamous epithelium and are normally expressed in the skin or esophagus and not the urinary bladder. In conclusion, our study shows that the genes involved in squamous differentiation, a basal subtype of muscle invasive bladder cancer are enriched in arsenite-transformed CICs and these cells give rise to tumors that show focal areas of squamous differentiation.

2322 Oncogenic KRAS Occurs after Prolonged In Vitro Arsenite Exposure of Human Prostate Epithelial Cells

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Inorganic arsenic is an environmental human carcinogen of the urinary tract that includes the prostate gland, which may be among its many target organs. RWPE-1 cells are immortalized human prostate epithelial cells and are non-tumorigenic, not forming tumors in nude mice and showing contact inhibition and anchorage dependence in soft agar assay. The Case-PE cell line was derived from prolonged (29 weeks), continuous, in vitro exposure of RWPE-1 cells to 50uM sodium arsenite. Case-PE cells are malignantly transformed according to the above three criterion but the mechanism remains unclear. We performed RNA-seq for differential gene expression and targeted sequencing to gain insight into in vitro arsenite transformation. RNA-seq showed 7,265 significantly altered transcripts in Case-PE cells compared to RWPE-1 cells at greater than 2-fold change. There were 3,261 up-regulated transcripts and 4,044 transcripts down-regulated transcripts. Pathway analysis of altered transcripts supported increased cell growth, cell motility and survival pathways in Case-PE cells. Notably, KRAS was increased over 400-fold and PSA (KLK3) rose over 50-fold. Connectivity analysis showed 34 annotated KRAS downstream transcripts were increased more than 5-fold, as well as an up-regulation of several growth factors including IGF1, IGF2, EGR1, VEGFA, and IGFBP7. Whole genome DNA sequencing of the KRAS gene revealed an allelic imbalance with high expression of a mutated transcript carrying an oncogenic mutation at codon 12 and many silent mutations, accompanied by relatively low expression of a wild-type (wt) allele. Parallel cultures of RWPE-1 cells retained a wt KRAS genotype. Copy number analysis and sequencing did not support amplification of a large genomic region containing the KRAS gene. Additional studies using 454 Roche sequencing reads showed KRAS variant switching in Case-PE transformed cells, where KRAS-4b was found as the predominant transcript variant, compared to the KRAS-4a variant that was primarily expressed in RWPE-1 cells and in a normal prostate epithelium donor. These data are consistent with KRAS driven proliferation pathways found in spontaneous tumors and transformed cell lines. It is well known that arsenite is not a direct mutagen. Thus, further investigation of the molecular and genomic events underlying increased expression and mutation of KRAS might help explain the ability of arsenite to malignantly transform RWPE-1 cells in this in vitro model exposure system.
Impacts of Gut Bacteria on Oral Bioaccessibility of Arsenic in Soils Using a Multi-compartment In Vitro Gastrointestinal Model

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The World Health Organization lists arsenic as one of the top 10 chemicals of major public health concern due to its high level of toxicity, worldwide prevalence, and exposure of millions of people around the world to elevated levels (>100 µg/L). Understanding the factors that regulate physiological absorption (i.e. bioavailability) of arsenic as well as arsenic effects on the gastrointestinal system upon oral ingestion can lend insight into exposure and potential ways to mitigate the potential for toxicity. For example, low pH of gastric fluids and metabolism of arsenic by gut bacteria have been found to decrease dissolution and absorption of inorganic arsenic in vitro. Different food consumptions of arsenic from food sources may cause different bioaccessibilities in the body, and after oral ingestion, the body’s response to these factors remains understudied. In this study, bioaccessibility of arsenic in two standard reference materials (i.e. sodium arsenate, hexavalent, and NIST 2710a) was assessed in a multi-compartment simulated gastrointestinal (GI) system consisting of a stomach, small intestine, and colon which contained gut bacteria sourced from the feces of female mice. For the arsenic bioaccessibility assay, soluble and insoluble arsenic were separated using a series of centrifugation and filtration steps. The concentrations of arsenic in each fraction were measured using neutron activation analysis. Colon bacteria from a mouse that had been exposed to arsenic before weaning were characterized using quantitative microbiology and 16S rRNA sequencing. For both reference materials, the bulk of the arsenic remained insoluble in all GI compartments. The gut bacteria community structure contained a host of microbes (e.g. Bacteroidetes, Firmicutes, Clostridia, Lactobacillus, and Proteobacteria), which was found in the human colon and the microbiota remained unchanged before and after acute arsenic exposure.

Chronic Exposure to Arsenic and High-Fat Diet Induces Sex-Dependent Renal Effects

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Obesity is one of the most common public health problems and it increases the risk of renal disease. Exposure to arsenic, a well-known global contaminant, is also associated with kidney disease. Chronic exposure to arsenic causes various health effects which may induce oxidative stress. There is a paucity of data on the combined effects of whole life, low-dose arsenic exposure and high fat diet (HFD) on the kidney. Additionally, gender differences in this response have not been explored. The present study aimed to determine the effects of combined arsenic and HFD on markers of kidney disease and gender-specific differences in the response. C57BL/6J mice were exposed to the 100 ppm arsenic via drinking water from conception to sacrifice. Dam mice were continuously exposed to arsenic before weaning. After weaning, mice were divided into HFD (42% calorie as fat) and normal or low fat diet (LFD, 13% calorie as fat) groups. At 10 and 24 wks, mice were sacrificed and kidneys harvested. We found HFD feeding increased body-weight gain compared to control. Exposure to arsenic slightly reduced HFD-increased body-weight gain in male mice, but further increased HFD-increased weight gain in female mice. Blood arsenic levels were very low without significant differences among groups at both time-points. There was no difference for renal arsenic levels between control and HFD-fed groups. However, As feeding significantly reduced renal arsenic accumulation in both sex groups at both time-points. Histological analysis showed that arsenic aggravated obesity-induced glomerular area expansion, mesangial matrix accumulation, and fibrosis. HFD induced renal inflammation and fibrosis, reflected by increased IL-1β, Lactate dehydrogenase, fibronectin, and CTGF. Arsenic exposure caused significantly increases in HFD-induced inflammatory and fibrotic effects, which were more pronounced in male mice. In female mice, there was a transient increase in antioxidant levels at 10 wks time-point, but significantly decreased at 24 wks in arsenic/HFD group. Therefore, we concluded that arsenic exposure caused time and sex-dependent alterations in HFD-induced kidney damage. In general, male mice had more severe responses than female mice.

The Gut Microbiota Modulates As-Disrupted Lipid and Cholesterol Homeostasis

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Arsenic (As)-induced LXR/RXR signaling inhibition is a potential mechanism underlying the cardiovascular effects caused by As. The gut microbiota can influence As toxic effects; however, whether it plays a role in As-induced LXR/RXR signaling inhibition and the subsequent lipid and cholesterol dysbiosis is still unknown. Here, we compared the As effects on the LXR/RXR signaling, lipid and cholesterol homeostasis in conventional mice and gut microbiota-disrupted mice. We investigated the hepatic gene expression by RNA-seq and we found As exposure significantly perturbed the LXR/RXR signaling axis and cholesterol synthesis pathway in conventional mice. After validated by qPCR, we found the expression of sreb1c, hmgcr, and cyp7a1 as well as that of cholesterol efflux genes, including abca1, abcg5, abcg8, and c63e, was inhibited by As exposure in conventional mice but not in AB-treated mice. Similarly, As exposure inhibited the hepatic expression of ldr and scarb1, which are involved in reverse cholesterol transport (RCT), in conventional mice. By contrast, 1 ppm As exposure increased the mRNA levels of ldr and scarb1 in AB-treated animals. Correspondingly, 1 ppm As exposure exerted opposite effects on the serum cholesterol levels in the two types of mice, increasing the serum cholesterol levels in conventional mice but decreasing these levels in AB-treated mice. Serum lipid levels, especially triglyceride (TG) levels, were increased in conventional mice exposed to 1 ppm As, while As exerted only minimal effects on the serum lipids in AB-treated mice. Liver lipid patterns were also differentially altered in the two types of mice exposed to 1 ppm As. In conclusion, gut microbiome disruption by antibiotics reverses the As-induced inhibition of the expression of downstream LXR/RXR signaling pathway genes and influences As-induced cholesterol and lipid homeostasis disorders. Therefore, the gut microbiota is a critical factor regulating As-induced LXR/RXR signaling perturbation, and modulation of the gut microbiota could be a promising intervention strategy to reduce the toxic effects of As on lipid and cholesterol homeostasis.

Building an Adverse Outcome Pathway Network for Arsenic-Induced Diseases

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Arsenic exposure has been associated with numerous diseases ranging from cancers of the bladder, skin and lung, to metabolic diseases such as cardiovascular disease and diabetes mellitus, as well as adverse pregnancy outcomes such as spontaneous abortions, low birth weights, and cognitive impairments of young children exposed either in the womb and or in early life. Multiple epidemiologic as well as animal studies have provided evidence that arsenic exposure can increase the human health risk for these diseases; however, the molecular events by which arsenic contributes to these diverse disease states is yet to be fully elucidated. Adverse outcome pathways (AOPs) are data-informed constructs that illustrate series of biological events leading to adverse effects. Our goal was to use the AOP framework to organize the abundance of information about arsenic-related diseases and identify important key events and possible knowledge gaps, specifically using a cancer example (bladder cancer) and a noncancer example (diabetes). To construct AOPs for these independent disease states, we performed a literature search in PubMed and identified peer reviewed medical reviews that focused on the molecular events involved in these idiopathic diseases. Using these publications we identified key events and key event relationships in the progression of these diseases and built the AOPs. We then compared these AOPs against the idiopathic disease pathways in the Kyoto Encyclopedia of Genes and Genomes (KEGG), and observed that our AOP constructs aligned well with the pathways in the KEGG database. We then began overlaying information from published arsenic mechanistic studies, and in doing so, we identified key events in the progression and culmination of arsenic-induced bladder cancer and arsenic-induced diabetes. This approach was helpful in informing susceptibility and identifying key mechanistic steps and data gaps in the onset of these two arsenic-related diseases. The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the US EPA. Mention of trade names or commercial products does not constitute endorsement or recommendations for use.
2327 The Role of O-GlcNAcylation in Driving Arsenic-Linked Metabolic Syndrome

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Chronic exposure to inorganic arsenic (iAs), mainly via contaminated drinking water, is linked to an enhanced risk of developing cardiovascular, kidney, and respiratory diseases, as well as some cancers and type II diabetes. Importantly, chronic iAs exposure can affect populations worldwide, from the United States, Canada, and Mexico, to Taiwan, China, and Bangladesh, where in many cases the iAs concentrations in the water significantly exceed the World Health Organization (WHO) recommended limit of 10 μg/L. Among the pathologies linked to prolonged exposure to iAs is metabolic syndrome, which is defined by an increase in a specific subset of metabolic risk factors, including obesity, dyslipidemia, insulin resistance and inflammation, all of which are associated with cardiovascular disease and type II diabetes. Key to the progression of metabolic diseases is a shift from normal homeostatic metabolism to a new metabolic program that is pro-pathogenic. As such, understanding the metabolic reprogramming that occurs in tissues affected during the progression of metabolic syndrome, particularly upon exposure to environmental toxins, is vital to enhancing current and future patient therapies. One key metabolic pathway is the hexosamine biosynthesis pathway (HBP), which is responsible for utilizing ~2-5% of the cell’s glucose to produce UDP-GlcNac, a key metabolite used in the O-GlcNAcylation of target proteins. Altered HBP activity, as well as significant changes in protein O-GlcNAcylation, have both been shown to contribute to the progression of metabolic syndrome. Here, we show that iAs treatment enhances global protein O-GlcNAcylation both acutely and chronically, with mass spectrometry data revealing a number of critical targets involved in glucose metabolism, mitochondrial function, vesicle trafficking, and proteostasis, among others. Differentiated NIH-3T3L1 adipocytes exposed to arsenic demonstrated significant changes in adipogenesis and insulin signaling compared to their control counterparts, with knockdown of OGT, the enzyme responsible for adding protein O-GlcNAcylation to proteins, partially rescuing the arsenic phenotype, inferring a key role for altered protein O-GlcNAcylation in driving arsenic-induced metabolic changes. Because iAs exposure increases the prevalence of disease in human populations, the effects of arsenic on the molecular, cellular, and pathophysiologic mechanisms that contribute to disease progression will prove extremely valuable in the generation of preventive and therapeutic strategies, as well as in the identification of biomarkers, for the general populations at risk.

2328 Arsenic-Induced Perturbations of the GR Pathway in Trophoblasts: Implications for Placental Dysregulation


Exposure to inorganic arsenic (iAs) is a public health issue as prenatal exposures to iAs are associated with adverse cognitive outcomes, low birthweight, spontaneous abortion, and infant mortality, among others. Previous research in non-placental cells has shown that iAs dysregulates the glucocorticoid receptor (GR) pathway. As this pathway is a critical regulator of cell signaling in the placenta, such dysregulation may contribute to the observed effects of prenatal iAs exposure. In this project, we set out to investigate whether iAs exposure modulates the expression of the GR signaling pathway in placental cells and a potential mechanism involved in this modulation. To investigate disruption of the GR pathway by iAs exposure, JEG-3 placental trophoblasts were treated with non-cytotoxic, environmentally-relevant doses of iAs (0.5-3 μM) for 24 hours and assessed for changes to GR-associated genes mRNA expression, protein expression, and DNA methylation. Results demonstrated that iAs treatment alters the expression of 12 GR-associated genes that play a role in nutrient transport, placental growth, cell survival, and embryonic implantation. These genes include Aquaporin 1 (AQPI), Period Homolog 2 (PER2), Metallothionein 2A (MT2A) and Serum-GC-regulated kinase 1 (SGK1), among others. Interestingly, seven of these 12 genes displayed a biphasic dose-response relationship, with low doses (0.5 μM) induced expression and high doses (3 μM) repressed expression. Treatment with iAs was not associated with changes in total trophoblast GR protein expression at any dose as determined by Western blot. Lastly, CpG methylation levels were also measured in trophoblasts after iAs treatment using the EPIC 850 K Illumina platform. Changes in DNA methylation levels were observed in all 12 genes that displayed changes in mRNA expression as a result of iAs treatment, suggesting a role for the epigenome in this altered mRNA expression. This study therefore supports prior reports that iAs acts as an endocrine disruptor and provides evidence of a novel mechanism by which prenatal iAs exposure may induce adverse birth outcomes.

2329 Metabolism of Arsenic in Genetically Diverse Collaborative Cross Mice


Mice have been preferred animal models for laboratory studies focusing on adverse effects of chronic exposure to inorganic arsenic (iAs). Like humans, mice metabolize iAs to form monomethylated and dimethylated metabolites (MAs, DMAs). However, mice metabolize iAs more efficiently than humans, resulting in a faster clearance and lower tissue retention. This may explain why some of the effects of iAs reported in humans have been difficult to reproduce in mice. The goal of this study was to find a mouse strain in which the metabolism of iAs resembles that in humans. Epidemiologic data suggest that the efficiency of iAs methylation depends in part on polymorphisms in AS3MT. The Collaborative Cross (CC) is a panel of recombinant inbred mouse strains designed to model genetic diversity of human populations. We examined iAs metabolism in male mice from 12 CC strains with different As3mt haplotypes or different levels of As3mt expression in the liver. The mice were exposed to iAs in drinking water (0.1 or 50 ppm) for one week and As metabolites were analyzed in urine and livers. We found significant differences in sum of As species and proportions (%) of iAs, MAs and DMAs, which were more pronounced in the liver as compared to urine, particularly in mice exposed to 50 ppm As. At this exposure level, large variations among the strains were found in hepatic %iAs (15-48%), %MAs (16-29%), and %DMAs (29-66%). In urine, DMAs represented 90-99% of total As in all CC strains regardless of exposure. In addition, %DMAs in urine did not correlate with sum of As species in the liver. Thus, the proportions of As species in urine were not representative of the internal exposure. Notably, As3mt mRNA expression in the livers of the CC mice exposed to 50 ppm correlated negatively with %iAs (R²=-0.35, P=0.013) and positively with %DMAs (R²=0.44, P=0.0014) in the liver. No significant correlations were found between As3mt expression and the proportions of As species in livers of mice exposed to 0.1 ppm, or in urine regardless of exposure level. Thus, we have not found yet a mouse strain in which proportions of As species in urine match those reported in human studies (10-30 %iAs, 10-20% MAs, 60-70% DMAs). However, the CC strains in which we observed low %DMAs in the liver after exposure to 50 ppm As (suggesting inefficient iAs methylation) may serve as better models for studies aiming to reproduce effects of iAs described in humans.

2330 Using the Zebrafish Model System to Define Developmental Arsenic Toxicity

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Arsenic (As) is an environmentally abundant environmental toxicant and in its inorganic form is highly toxic. High levels of As in soil can dissolve into water, contaminating ground water, the most common source for human exposure. Both the US EPA and the Department of Health and Human Services (DHHS) have determined that both acute and chronic exposure to high levels of inorganic As can cause cancer; however, evidence is increasing that chronic exposure to As in children may also be associated with disruption to cognitive development and function. While the mechanism of action of As is not completely understood, As at low levels can cause a range of cardiovascular and circulatory health effects that can directly and indirectly translate to insults of the brain. This study tests the hypothesis that embryonic exposure to As results in specific changes to survival, behavior, and morphology of larval zebrafish. Zebrafish embryos were treated with two concentration ranges of As (Sodium Arsenite) [0, 0.1, 0.5, 1, 5, 10 μM] or [0, 0.05, 0.1 mM] immediately after fertilization and exposed through 120 hours post fertilization (hpf). The higher concentration range was first used to calculate LC50s every 24 hours for As during the developmental time period through 120 hpf. The lower concentration range was then applied to assess impacts to behavior using the visual motor response (VMR) assay and morphology including total larval length, head length, head width, and brain length at 120 hpf. The LC50s were calculated at 3.2 mM, 3.4 mM, 2.5 mM, 1.1 mM, and 1.7 mM at 24, 48, 72, 96, and 120 hpf, respectively. The larval VMR results indicate that the 0.1 mM treated larvae were hypoactive (p<0.05). Significant differences were also observed at the morphological endpoints in the 0.1 mM treatment group (p<0.05). This study will set the stage for further exploration of the influence of As in mixtures with other metals.
2331 Metabolic Phenotype of Genetically Diverse Collaborative Cross Mice Exposed to Arsenic


Arsenic methyltransferase (As3mt) is the key enzyme in the pathway for methylation of inorganic (iAs) and organic arsenic. Alternative splicing pathways play an essential role in detoxification of iAs. As3mt converts iAs to mono- and dimethylated metabolites (MAs, DMAs) that are excreted mainly in urine. Polymorphisms in As3mt have been identified as the single most important genetic factor affecting iAs metabolism and susceptibility to adverse effects of iAs exposure in population studies. However, the relationship between As3mt polymorphism and the adverse outcomes of iAs exposure have never been examined in animal models. The goal of the present study was to examine effects of iAs exposure on metabolic phenotypes of mice from two Collaborative Cross strains, CC021/Unc (CC021) and CC027/ TauUnc (CC027), that have different As3mt haplotypes (NOD/ShiLtj and WSB/Eii, respectively) and different SNPs in As3mt gene. The patterns of iAs metabolism in these two strains, specifically the proportions of total arsenic present as iAs (%iAs) and DMAs (%DMAs) in the liver and urine, are also significantly different. Here, the male mice from the two strains were exposed to iAs in drinking water (0.1 or 50 ppm) for 11 weeks. Blood glucose and plasma insulin levels were measured after 6-hour fasting and 15 min after ip. injection of glucose (2 g/kg b.w). Body composition (%fat and %lean mass) were determined using MRI. Prior to iAs exposure, fasting glucose was higher and insulin lower in CC027 as compared to CC021 mice. iAs exposure had no statistically significant effects on either glucose or insulin measures. However, it affected the body composition and the effects differed between the strains. After exposure to 50 ppm iAs, %fat was ~3-fold higher in CC021 as compared to CC027 mice, while %lean mass was lower (p<0.05). Similar differences were found in mice exposed to 0.1 ppm iAs, but these differences did not reach statistical significance. No statistically significant differences were found in body composition of the unexposed CC021 and CC027 controls. CC021 mice had significantly higher fasting and 15-min plasma insulin levels than CC027 mice regardless of the treatment. These results suggest that iAs exposure influences adiposity of CC021 and CC027 mice, but the direction of these effects may depend on As3mt genotype and/or on other genetic characteristics of these mouse strains.

2332 Sexually Dimorphic Hepatic Responses to Environmental Arsenic Exposure in a Mouse Model of Non-alcoholic Fatty Liver Disease

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Obesity is a major risk factor for the development of non-alcoholic fatty liver disease (NAFLD), but progression of the disease is dependent on additional factors. Recent studies suggested that inorganic trivalent arsenic (arsenite) is such a factor. The purpose of the present study was to delineate pathways that are altered by arsenic in a mouse model of NAFLD. Male and female C57BL/6J mice were continuously exposed to 100 ppm arsenite in drinking water starting 2 weeks before conception, and this was combined with consumption of a Western-style high fat diet (HFD) after weaning for up to 6 months. Normal drinking water and defined low fat diet (LFD) were used as controls. Changes in liver pathology, redox status and gene expression were assessed at 10 and 24 weeks after weaning. Consumption of HFD for 6 months resulted in obesity and steatosis (fatty liver) in both male and female mice. In mice fed LFD, arsenite exposure had no observable effect in males or females. The HFD produced more severe liver injury in male mice than in female mice. Females gained more weight with the combination of arsenic and HFD than they did on HFD alone, an effect not seen in males. RNA-seq analysis was performed at 10 weeks after weaning. In female mice, 76 genes were differentially expressed in response to arsenite in the context of HFD. Of these, 12 (16%) encoded xenobiotic metabolizing enzymes. In male mice, 26 genes were differentially expressed. Five of these (19%) were genes involved in immunity and inflammation. There were no differences in antioxidant gene expression, hepatic glutathione/glutathione disulfide levels, or plasma cytokine/cytokine concentrations. Sex-specific differences in hepatic gene expression and consumption of different diets contributed to differences in the ways liver injury is manifested in response to whole-life exposure to environmental arsenic.

2333 Alternative Splicing Events and RT-PCR Analysis of SHC1 in Arsenic Exposed Human Keratinocytes

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Chronic arsenic exposure in drinking water is associated with an increased risk of developing skin, lung and urinary bladder cancer. The molecular etiology of arsenic induced cancer remains elusive. Pre-mRNAs are often subject to alternative splicing that either includes or excludes exons in the mature mRNA resulting in synthesis of functionally distinct protein isoforms. The imbalance in isoform species can result in pathogenic changes in critical signaling pathways. Arsenic exposure has been reported to induce differential mRNA splicing. Differential RNA splicing was investigated in a model of skin carcinogenesis to test the hypothesis that alternative splicing events induced by arsenite exposure play a role in skin carcinogenesis. Multiple cultures of HaCaT cells, 4 each with 0 (As-) or 100 (As+) nM NaAsO2, were maintained for 7 weeks. We mapped 100x2bp paired-end RNA-seq reads to the human genome (hg38) and transcriptome (Ensembl, release 82) using the software STAR (v2.5) allowing up to 3 nt mismatches per read and up to 2 nt mismatches per 25 nt seed. To identify differential arsenic events between the As- and As+ samples, we used rMATs v3.2.5 to identify differential alternative splicing events from strand specific RNA-seq data corresponding to five basic types of alternative splicing patterns. As+ group was compared to As- to identify differentially spliced events with an associated change in percent-spliced-in (ΔPSI) of these events. Skipped exons (SE) with > 10 event readings and mean ΔPSI > 30% were analyzed. Based on functional relevance, Src homology 2 domain containing adapter protein 1 (SHC1) was selected for validation by RT-PCR. 1,149 SE events were significantly different between As- and As+ 66 mRNA isoforms - and 61 genes - showed > 10 event readings and mean ΔPSI > 30%. For SHC1, RNA-seq predicted SE counts decreased in As+. However, the RT-PCR did not detect significant changes between the two isoforms. Arsenic exposure causes alternative splicing events that might impact disease pathology. Further SHC1 sequencing and protein validation, and investigation of other alternative spliced targets would be essential to elucidate the mechanisms involved in arsenic-induced skin carcinogenesis. Funding: NIH grants R01ES027778, R21ES023627, P20GM103436, R15GM126446.

2334 Investigating Cell Type Specific immune Responses to Acute Influenza A Infection in Adult Mice Exposed to Arsenic In Utero

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Exposure to inorganic arsenic (iAs) via drinking water is associated with numerous adverse health effects, including lung disease, and impacts hundreds of millions of people worldwide. iAs exposure early in life is of particular concern and has been linked in epidemiological studies with increased respiratory infections during infancy, as well as increased morbidity and mortality from non-malignant lung diseases in adulthood. Studies in mouse have similarly found that iAs exposure increases morbidity from respiratory virus infection. We examined changes in immune cell populations and gene expression to investigate the effects of iAs exposure on cell-type specific gene expression and immunopathologic responses during adulthood. Pregnant dams were given Control or 100 µg/L iAs in drinking water ad libitum. Upon birth, iAs was removed from the drinking water and pups matured to adulthood (>7 weeks of age), when they were inoculated with a sublethal IAV infection. We examined changes in cell populations and gene expression in the lung using single cell RNA sequencing (sc-RNA-seq), and assessed inflammatory response and damage by measuring cytokines, immune cell infiltration, and albumin in bronchoalveolar lavage fluid (BALF). Mice exposed to iAs in utero had significantly altered immune cell populations in the lung, as detected by sc-RNA-seq, which correlated with significantly increased immune cells and IL1β in the BALF. We then used cell-type specific gene expression data to predict signaling networks that mediate the hyperinflammatory response. These data provide insight into molecular changes in immune cells that result from in utero iAs exposure and may mediate lasting predisposition to respiratory disease.
Differential Gene Expression Patterns in Subcutaneous and Intra-peritoneal Tumors Produced by Urotsa Cells Malignantly Transformed by Cadmium and Arsenite


Arsenite (As³⁺) and cadmium (Cd²⁺) are environmental toxicants that are associated with the development of various cancers, particularly urothelial cancers. These cancers spread locally requiring the ability of tumor cells to colonize the peritoneal organs following escape from the bladder. This laboratory has developed an in vitro model of bladder cancer by exposing the immortalized urothelial cell line, UROtsa, to As³⁺ and Cd²⁺ resulting in the generation of As³⁺- and Cd²⁺-transformed lines and seven Cd²⁺-transformed cell lines. Of these transformed cell lines three of them (As Name 1, As Name 2, and Cn Name 1) can form tumors in the peritoneal cavity (IP), whereas the rest of the transformed lines form only subcutaneous (SQ) tumors. The goal of this study was to determine if there was a difference in the gene expression patterns of the SQ tumors versus the IP tumors. The histology of the SQ and the IP tumors was similar with the exception of the degree of squamous differentiation, which was significantly lower in the IP tumors when compared to the SQ tumors. There was a decrease in the expression of the keratins (KRTs) 1, 6, 10, 14, 16, and 17 in the IP tumors when compared to the SQ tumors. However, the distribution of the expression of some of the keratins was different between the SQ and the IP tumors. KRT17 was mainly expressed in the well-differentiated areas of the SQ tumors, while expression in the IP tumors was found in the well-differentiated as well as the less-differentiated areas of the tumors. The expression of KRT19 was lower in the well-differentiated areas of the SQ tumors when compared to the IP tumors. In conclusion, the reduced expression of KRTs in the IP tumors suggests that KRTs may play a role in the metastatic spread of the tumors and their reduced expression may facilitate the process of metastasis.

The CD24⁺CD133⁺ Cells Isolated from Human Proximal Tubules Have Proliferative and Regenerative Potential in Response to Insult by the Environmental Toxicant Cadmium


The proximal tubules of the kidney are a major site of toxic insult, cell death and regeneration, and the development of renal tubular diseases. Toxic insult can result from exposure to heavy metals, pharmaceuticals, diabetic induced nephropathy and ischemia. Cadmium an environmental nephrotoxicant accumulates in the proximal tubule cells leading to overt renal damage. Previous studies from our laboratory have shown that the human proximal tubules contain a population of cells co-expressing the cell surface markers CD24 and CD133. This CD24⁺CD133⁺ double positive population is scattered throughout the kidney cortex and is capable of regenerating. Under cell culture conditions, these cells can be expanded and they retain their phenotype and their potential for self-renewal as well as the capacity to differentiate. The goal of this study was to determine the response of this progenitor population to the environmental toxicant cadmium and determine their ability to regenerate and differentiate. Our results suggest that the CD24⁺CD133⁺ proximal tubule cells are more resistant to the toxic effects of cadmium with increased ability to proliferate and regenerate in response to cadmium exposure when compared to the cells that only express CD24, which are more sensitive and lack the proliferative capacity. In addition, the CD24⁺CD133⁺ cells show in vitro tubulogenic, osteogenic, adipogenic and neurogenic differentiation, whereas the CD24⁺ cells lack the ability to differentiate. In conclusion, our study demonstrates that the CD24⁺CD133⁺ cells are the progenitor/stem cells that have proliferative and regenerative capabilities and exhibit multi-lineage differentiation potential, whereas the CD24⁺ cells are the differentiated cells that are sensitive to toxic insults and lack proliferative and regenerative capacities.

Environmental Cadmium Exposure Causes Lung Injury by Dysregulation of Mitochondrial Mechanisms

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Activation of oxidative stress mechanism is commonly associated with toxicity from environmental metals yet delineation of effects of low-dose metal exposures is complicated by positive and negative interactions with dietary factors, infection and other factors. Our previous study showed low-dose cadmium (Cd) toxicity in lung with potentiation of adverse Cd-dependent mechanisms following viral infection, and prevention of low dose Cd toxicity through nutritional selenium supplementation. In the present study, we examined the impacts of Cd burden at a low environmental level on lung metabolism, redox proteome, and inflammation in mice given Cd by drinking water (3.3 mg/L) for 16 weeks. The results showed that mice accumulated lung Cd comparable to non-smoking humans and showed increased levels of lipids in the lung, accompanied by disruption in mitochondrial energy metabolism. In addition, targeted metabolomic analysis showed that Cd-treated mice had increased accumulation of mitochondrial carnitine and citric acid cycle intermediates. The results of redox proteomics showed that Cd-stimulated oxidation of isocitrate dehydrogenase, malate dehydrogenase and ATP synthase. Taken together, our study shows that low dose Cd-enhanced lung inflammation is mediated by the mechanism involving impaired mitochondrial function and oxidative stress. These findings suggest that dietary Cd intake relevant to non-smokers without occupational exposures could be an important variable contributing to human pulmonary disorders.

Prenatal Exposure to Cadmium (Cd) and Postnatal Exposure to Cd and High-Fat Diet (HFD) Impairs Spermatogenesis and Increases Testicular Apoptosis

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Obesity is a global health problem that negatively impacts testosterone levels and the quality of sperm production. The persistent environmental toxicant Cd also affects testicular tissues. The effects of prenatal and postnatal Cd exposure on spermatogenesis and the ability of HFD to further disrupt this process were evaluated. C57BL/6J mice were exposed to the low-dose (L-Cd, 0.5 ppm) or high-dose (H-Cd, 5 ppm) Cd via drinking water from conception to sacrifice. Offspring were continuously given two doses of Cd and fed either normal (NFD, 13% calorie as fat) or HFD (42% calorie as fat) once weaned for 10 and 24 wks and then sacrificed. Testes were harvested, weighted and then processed for metal analysis and histopathology. All round seminiferous tubules per animal were examined for germ cell degeneration and spermatogenesis. Apoptotic death in seminiferous tubules was analyzed by TUNEL staining. Data were analyzed by ANOVA. Exposure to H-Cd and HFD alone caused a significant increase in Cd content in testis. There was also a significant Cd-diet interaction. There was no difference in the number of round seminiferous tubules among animals fed NFD exposed to L-Cd or H-Cd. HFD induced high incidence of seminiferous tubule sloughing. There was an age effect on the incidence of multinucleated large cells within the lumen (24 > 10 wks old mice), which was not affected by Cd or diet. There were significant increases in apoptotic testicular cell death and detached seminiferous tubules, as well as decreased spermatogenesis in animals exposed to H-Cd at 24 wks. HFD caused central germ cell degeneration. These results suggest that chronic exposure to Cd impairs spermatogenesis in mice independent of diet, which may link environmental Cd exposure to ongoing unexplained male infertility.

SPARC Expression at Tumor Initiation and after Tumor Formation in a Bladder Urothelial Carcinoma Model System


Bladder cancer has a strong link to environmental exposure to toxicants. Our system uses this link to model urothelial carcinoma due to heavy metal cadmium (Cd) exposure to examine potential biomarkers. UROtsa bladder cells exposed to long-term, low doses of Cd were studied in 7 independently malignantly transformed cell lines. The resulting microarray analysis identified SPARC as the most repressed gene. SPARC, a secreted protein, has both oncogene as well as tumor suppressor actions depending upon the cancer studied with its role in bladder cancer remaining unclear. Previous lab results indicate significant SPARC repression following even short-term exposure to Cd and SPARC mRNA and protein is barely detectable, if at all, in the 7 Cd-transformed cell lines. Therefore, SPARC was transfected back into the Cd-transformed lines and their ability to form heterotransplant tumors was determined. SPARC was again repressed to undetectable limits in tumor heterotransplants. However, when corresponding stem cell UROospheres were injected, some SPARC expression was maintained in a small percentage of the tumor. In this study,
serial heterotransplant tumors were generated and results showed SPARC expression was not maintained nor increased within the tumor cells, even with successive tumor inoculations of the UROsphere generated tumors. However, stromal SPARC expression was found to increase. Therefore, we hypothesize that SPARC is inhibited within the tumor cells themselves and possibly by high stromal SPARC expression. The origin of SPARC stromal expression in heterotransplants was determined using RT-PCR with human or mouse specific primers. Results show that nearly all the SPARC in the heterotransplants was of mouse and not human origin, indicating that the mouse tumor stroma and not the human tumor cells were responsible for the SPARC expression. Further in vitro experiments showed that SPARC expression in the cell lines was repressed when exposed to exogenous human or mouse SPARC. Additional studies include examining transcription factors in SPARC repression and the role of SPARC in cell attachment and spreading. Overall, SPARC expression appears to play a vital role in tumor formation where high stromal SPARC may repress expression by tumor cells.

2340 Disposition and Toxicity of Cadmium in Wild-Type and BCRP-Null Mice Following Acute Exposure

Cadmium (Cd) is a highly toxic, present in tobacco smoke, paint pigments, Cd-Ni batteries, and fertilizers. Upon exposure, the metal accumulates in multiple tissues including the liver, kidney, testes, and placenta where it induces cellular stress and interferes with hormone production and nutrient homeostasis. The breast cancer resistance protein (BCRP) is an efflux transporter expressed in liver, kidneys, testes, and placenta. Our laboratory has demonstrated that Cd exerts an in vitro overexpression of the human BCRP gene down-regulation. In order to study the effects of Cd on enzymes involved in the detoxification process, the activities of enzymes catalase (CAT) and superoxide dismutase (SOD) were examined by Pearson’s method, revealed positive association between CAT, and SOD triglyceride concentrations compared to control. Correlation, as calculated by Pearson’s method, revealed positive association between CAT, and SOD triglyceride; SOD and plasma triglyceride; as well as ALP and ALT, respectively. The antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) activities were significantly (p < 0.05) decreased in rat exposed to the toxicants. Induction of Cd2+-cytotoxicity, a response attenuated by pharmacologic inhibition of BCRP. However, it is unknown whether a similar relationship between BCRP and Cd2+ toxicity occurs in vivo. In the current study, we sought to assess the disposition and toxicity of Cd2+ following acute exposure. A single dose of vehicle or CdCl2 (5.5 mg/kg ip) was administered to adult male and female wild-type (WT) and Bcrp-null mice (n=3-6). Tissues were collected at 24 hr for histopathologic evaluation and ICP/MS quantification of Cd levels. There were no noteworthy histopathologic findings in the kidneys or testes from WT and Bcrp-null mice. However, evidence of Cd-induced apoptosis was observed in the livers of Bcrp-null mice. Cd was detectable in all tissue samples from CdCl2-treated mice. At 24 hr, concentrations of Cd were highest in the liver and lowest in the testes with liver and kidney concentrations similar between male and female mice. Interestingly, there was a 30% higher accumulation of Cd (p=0.05) in the kidneys of male Bcrp-null mice compared to WT mice. Additional dose-response and time course studies are being conducted to further determine whether Cd2+ exposure and toxicity differ between WT and Bcrp-null mice. Supported by R01 ES029275, T32 ES007148, and P30 ES005022.

2341 Exposure to Lead and Cadmium Contaminating Metals: A Biochemical, Oxidative Stress and Lipid Profile Study in a Rat Model
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Heavy metals are dangerous environmental bio-accumulative pollutants that affect many organs in human and animals leading to a broad range of physiological, biochemical, and neurological dysfunctions. The study investigated the effect of 0.1 ml per body weight cadmium (cadmium chloride) and lead (lead nitrate) via drinking water on oxidative stress, lipid profile and biochemical parameters of four groups of male rat for thirty days. Control animals received distilled water for the same period. Alanine aminotransferase (ALT), aspartate aminotransferase and alkaline phosphatase concentrations were significantly (p < 0.05) increased by the treatment of cadmium (Cd), lead (Pb) and mixture of both cadmium-lead (Cd-Pb) respectively. The antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) activities were significantly (p < 0.05) decreased in rat exposed to the toxicants. Induction of Cd and Pb reduced significantly (p < 0.05) the plasma cholesterol and triglyceride concentrations compared to control. Correlation, as calculated by Pearson’s method, revealed positive association between CAT, SOD and plasma triglyceride; SOD and plasma triglyceride; as well as ALP and ALT, AST and plasma cholesterol; while negative association exists between CAT and AST activities. These results demonstrate that Cd, Pb and Cd-Pb induces oxidative and metabolic disturbance which might lead to potential risk of cellular dysfunctions.

2342 Systematic Review on the Effects of a 0.5 μg/dL Blood Lead Level in Children and Pregnant Women
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Presently, no safe blood lead level (BLL) in children has been identified. We conducted a systematic review of published, peer-reviewed epidemiological studies to determine whether a BLL of 0.5 μg/dL, which corresponds to dietary lead intake of 3 μg/day in children (aged 0-6) and 12.5 μg/dL in women of childbearing age, is associated with adverse health effects in pregnant women, their offspring or children of all ages. A review article from Health Canada provided the basis for literature prior to 2010, and PubMed and Web of Science were utilized to search from 2010 to February, 2018. We limited our review to studies examining study populations with mean BLL ≤ 20 μg/dL to reduce the influence of high BLL on the results. Review articles, occupational, inhalation, or dermal exposure, neurodevelopmental, in vivo animal, in vitro, in silico, or foreign language studies were excluded. Studies examined the relationship of BLL with several different outcomes, including, but not limited to anthropometric measurements, APGAR score, blood lipids, blood pressure, cardiovascular function, caries, cytogenetic damage, gestational age, hearing loss, hemoglobin, hormone levels, insulin resistance/blood glucose, kidney function, oxidative stress or pertubal onset of children; maternal pregnancy outcome, blood pressure, cardiovascular function, or mental status; and placental weight. Although some studies reported associations between ranges of BLL that included 0.5 μg/dL and changes in endpoints, none clearly identified a BLL of 0.5 μg/dL as an adverse effect level.

2343 Comparing the Relative Bioavailability (RBA) of Lead (Pb) in Soil and Dust

Soil and dust are the predominant sources of Pb exposure for children in the United States. Although quantitation of the intake of Pb from soil and dust ingestion is problematic, the importance of these sources is emphasized by studies that show that reducing levels of Pb in soil and dust lowers blood Pb levels in children. Here, we have used a mouse assay to estimate the RBA for Pb in urban soil and dust. These studies used NIST Standard Reference Materials (SRM) with certified mass fraction values for Pb as test materials. SRMs tested were 2584 (indoor dust), 2587 (soil containing Pb from paint), and 2710a (Montana I soil). Pb acetate (PbAc) was used as a source of highly soluble Pb. The test materials were used to amend AIN-93G rodent diet that was consumed by adult female C57BL/6 mice in an established assay for estimation of Pb bioavailability. Final levels of Pb in test diets ranged from about 1 to 30 parts per million. RBA estimates for Pb in different test materials were derived from linear regression analysis of the relation between cumulative Pb intake from amended diet and the concentration of Pb in skeleton or blood, two tissue biomarkers of Pb exposure. For blood, the rank order of slopes of regression lines from highest to lowest was 2584 > PbAc > 2587 > 2710a. For soil, the rank order of slopes of regression lines from highest to lowest was PbAc > 2584 > 2587 > 2710a. Thus, the rank order of RBA estimates derived from blood is 2584 > 2587 > 2710a. The rank order of RBA estimates derived from skeleton is 2584 > 2587 > 2710a. Differences in the RBA for Pb in different soil and dust samples may reflect differences in the speciation of Pb present in these materials or modifying effects of constituents that are present in some environmental media. Continuing studies of matched soil and dust samples collected from individual homes will provide new insights into the relation between sample composition, Pb speciation, and bioavailability. This abstract does not reflect US EPA policy.

2344 A Rare Case of Severe Life-Threatening Lead Poisoning Due to Accidental Exposure: Diagnosis, Treatment, and Prognosis
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Chronic long-term, low-dose environmental exposure to lead (Pb) has been extensively studied in large cohorts throughout the world. Clinical cases of occupational Pb exposure have also been reported among miners, smelters and battery workers. However, acute and severe Pb intoxications due to accident
exposure that threaten human life are rarely seen in literature. Here we report one clinical case administered to the emergency room in Beijing Chaoyang Hospital with life-threatening Pb intoxication. A 27-year-old woman initially complained menstrual pain and was prescribed a powdered Chinese medicine. The woman, however, was instead mistakenly provided with the pure Pb powder. Two hours after taking 12 grams Pb powder, she suffered from abdominal pain, vomiting, and diarrhea. Laboratory examinations revealed high lead levels in blood and urine. She was treated with hemofiltration, followed by EDTA chelation for six courses (3-day EDTA/4-day pause). After treatment, anemia and liver dysfunction were recovered, BLL and ULL turned to normal, and majority clinical symptoms relieved. In another case, a 42-year-old man worked in a lead and manganese (Mn) smelting factory for 5 years. He was brought to the emergency room with severe nausea, vomiting, colic, and irritability. He presented chronic encephalopathy, anemia look and accompanied with the right shoulder pain. BLL and ULL were 64.8 μg/dL and 0.383 mg/L, respectively; however, both blood and urinary Mn were normal. EDTA chelation increased urinary Pb excretion and improved clinical symptoms at 1 day. Yet high patients signed an informed consent and completed a health survey. This study was approved by the Ethics Committee of the University of Cartagena. The K-BIT intelligence test was administered to measure the IQ; blood Pb levels (BLL) were evaluated by graphite furnace atomic absorption spectroscopy; whole blood was used for hematology; gene expression was quantified from blood mRNA using RT-PCR; The ALAD polymorphism (rs 1800435) was characterized by PCR-RFLP. The BLL average was 3.3±0.2 μg/dL; BLLs above the CDC reference level (5 μg/dL) were found in 15.3% of studied samples. The highest BLL were detected in the fishing community of Tasajera, where the 97.5th percentile was 22.1-fold greater than the national value reported for the US. Being male, maternal breastfeeding and natural childbirth were associated with greater BLLs. Spearman correlation analysis showed negative associations between BLL and IQ, weight, stature, BMI, neutrophil-basophil and neutrophil-eosinophil ratios, whereas positive correlations appeared for BLL and platelets, monocytes and neutrophil-monoocyte ratio. The expression of ADAM, SOD, p53 and INF-γ mRNA decreased with Pb exposure, whereas TNF-α mRNA increased. BLL were not significantly different with respect to the ALAD genotypes. In short, Pb exposure impacts IQ, physical development, hematological markers and cytokine gene expression in children from the Colombian Caribbean. Here, we present evidence that these techniques produce results equivalent to the colony filter lift assay. And that the intensity of the blue color is related to the concentration of lead. Previously, we relied on replica plating to propagate yeast colonies. However, in this study we bloated and dried yeast two-hybrid colonies onto filter paper. Despite the stresses placed on the yeast, once the filters were placed on normal media, they experienced a full recovery with the system intact. Traditionally, the use of liquid nitrogen is required for the colorimetric system to function. We provide two different methods instead: the use of a standard freezer or dry ice combined with ethanol. This allows for ease of use of the system for those without specialized equipment or technical knowledge. Furthermore, the system can be grown on lead concentrations as high as 10,000 ppm without loss of function.

2345 Lead Impairs Intellectual and Physical Development, Platelet Number, Gene Expression of Cytokines, and Oxidative Stress Status in Children from the Colombian Caribbean
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Lead (Pb) is a neurotoxic element that affects children health, especially those living under poverty. The objective of this research was to evaluate Pb exposure and its relationship with IQ, hematological markers and gene expression in children from the Colombian Caribbean. A total of 908 blood samples were collected from participants between 5 and 16 years of age. Yet high levels of Pb were found. The ALAD polymorphism (rs 1800435) was characterized by PCR-RFLP. The BLL average was 3.3±0.2 μg/dL; BLLs above the CDC reference level (5 μg/dL) were found in 15.3% of studied samples. The highest BLL were detected in the fishing community of Tasajera, where the 97.5th percentile was 22.1-fold greater than the national value reported for the US. Being male, maternal breastfeeding and natural childbirth were associated with greater BLLs. Spearman correlation analysis showed negative associations between BLL and IQ, weight, stature, BMI, neutrophil-basophil and neutrophil-eosinophil ratios, whereas positive correlations appeared for BLL and platelets, monocytes and neutrophil-monocyte ratio. The expression of ADAM, SOD, p53 and INF-γ mRNA decreased with Pb exposure, whereas TNF-α mRNA increased. BLL were not significantly different with respect to the ALAD genotypes. In short, Pb exposure impacts IQ, physical development, hematological markers and cytokine gene expression in children from the Colombian Caribbean. Here, we present evidence that these techniques produce results equivalent to the colony filter lift assay. And that the intensity of the blue color is related to the concentration of lead. Previously, we relied on replica plating to propagate yeast colonies. However, in this study we bloated and dried yeast two-hybrid colonies onto filter paper. Despite the stresses placed on the yeast, once the filters were placed on normal media, they experienced a full recovery with the system intact. Traditionally, the use of liquid nitrogen is required for the colorimetric system to function. We provide two different methods instead: the use of a standard freezer or dry ice combined with ethanol. This allows for ease of use of the system for those without specialized equipment or technical knowledge. Furthermore, the system can be grown on lead concentrations as high as 10,000 ppm without loss of function.

2346 Use of Zea mays Cob (Corn cob) as an Economic Adsorbent for the Adsorption of Lead (II) Ions from Aqueous Solution
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The increase in industrial activity is contributing to the significant upswing of potential toxic metals (PTMs) pollution in water resources. The PTMs thereby constitute a threat on the land and aquatic life. This study entails the use of corncob (CC), a biopolymer to absorb lead (II) ions from aqueous medium by batch process. The CC was characterised using analytical techniques: Fourier transform infrared (FTIR), scanning electron microscopy (SEM), thermogravi- metry analysis (TGA), x-ray diffraction (XRD) and point of zero charge (PZC). The pH, CC dosage, initial Pb (II) ions concentration, contact time, and temperature were optimized. The optimum pH was achieved at 3.00, with adsorp- tion of 4.64 mg/g. The kinetic data fitted better to pseudo-second order, and the accuracy was tested by three statistical tools: sum of square error, Chi-square and normalized standard deviation. The experimental data was applied to five isotherm model: Langmuir, Freundlich, Temkin, Dubinin- Kaganer-Raduskevich (DKR) and Halsey. DKR and Temkin best described the equilibrium adsorption data with regression coefficient (R2> 0.80). The ther- modynamic parameters values of standard enthalpy (ΔH°) 4.00 KJ/mol, standard entropy (ΔS°) 4.10 J/mol K and the Gibb free energy change revealed that the adsorption process was endothermic, spontaneous and feasible in nature. The results highlight the potential capability of corn cob in the effective removal of lead ions from aqueous solution.

2347 Development and Use of a Yeast Two Hybrid System to Detect Lead
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Despite advances, lead contamination continues to be an issue. This includes industrial sectors and perhaps more impactfully, lead contamination in residential areas which includes schools. We propose a methodology of lead detection using yeast as a living biodetector; a simple organism that can be easily grown and maintained in multiple environments. Here we use yeast strains that contain the yeast-two hybrid system, first developed by Fields and Song. In brief, this system is used to identify new protein-protein interactions. The system has been altered to be used as colorimetric biological detector of lead. With properly implemented controls, one can identify the presence of lead (Pb) in a simple media mixture through a visual readout. Here, we use a LacZ reporter system to facilitate the colorimetric indicator. The colorimetric indicator used in the system changes yeast colonies from blue to white when lead is present in the yeast. Here, we present evidence that these techniques produce results equivalent to the colony filter lift assay. And that the intensity of the blue color is related to the concentration of lead. Previously, we relied on replica plating to propagate yeast colonies. However, in this study we bloated and dried yeast two-hybrid colonies onto filter paper. Despite the stresses placed on the yeast, once the filters were placed on normal media, they experienced a full recovery with the system intact. Traditionally, the use of liquid nitrogen is required for the colorimetric system to function. We provide two different methods instead: the use of a standard freezer or dry ice combined with ethanol. This allows for ease of use of the system for those without specialized equipment or technical knowledge. Furthermore, the system can be grown on lead concentrations as high as 10,000 ppm without loss of function.

2348 Inhibition of Insulin Secretion by As, Cd, and Mn Is Associated with Metal-Specific Shifts in miRNA Profiles
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Type 2 diabetes (T2D) is a complex metabolic disorder characterized by hyperglycemia. While diet and exercise can influence the risk of T2D, chemical exposures have also been shown to impair mechanisms regulating glucose homeostasis and thereby increase T2D prevalence. Laboratory and population studies have shown that exposure to inorganic arsenic (iAs) impairs insulin secretion from pancreatic β-cells and leads to fasting hyperglycemia and/ or impaired glucose tolerance. Exposures to other metals, including cadmium (Cd) and manganese (Mn), have also been linked to T2D phenotypes, but data on the effects of these metals on insulin secretion from pancreatic β-cells is limited. In this study, we used a reporter system to monitor the secretion of insulin from yeast strains that contain the yeast-two hybrid system, first developed by Fields and Song. In brief, this system is used to identify new protein-protein interactions. The system has been altered to be used as colorimetric biological detector of lead. With properly implemented controls, one can identify the presence of lead (Pb) in a simple media mixture through a visual readout. Here, we use a LacZ reporter system to facilitate the colorimetric indicator. The colorimetric indicator used in the system changes yeast colonies from blue to white when lead is present in the yeast. Here, we present evidence that these techniques produce results equivalent to the colony filter lift assay. And that the intensity of the blue color is related to the concentration of lead. Previously, we relied on replica plating to propagate yeast colonies. However, in this study we bloated and dried yeast two-hybrid colonies onto filter paper. Despite the stresses placed on the yeast, once the filters were placed on normal media, they experienced a full recovery with the system intact. Traditionally, the use of liquid nitrogen is required for the colorimetric system to function. We provide two different methods instead: the use of a standard freezer or dry ice combined with ethanol. This allows for ease of use of the system for those without specialized equipment or technical knowledge. Furthermore, the system can be grown on lead concentrations as high as 10,000 ppm without loss of function.
context of IAs treatment is mir-146a (nearly 2-fold), a known transcriptional target of Nuclear factor xB (NF-xB). Cd and Mm exposure both disrupt the expression of miRNAs shown to be involved in oxidative stress responses. We propose that dysregulation of these miRNAs may be partially responsible for the inhibition of GSIS after metal exposure. We are currently performing studies in INS-1 B2/23 cells to determine whether miRNA over-expression or suppression can mitigate the negative effect of metal exposure on GSIS. Results of this study will help characterize the roles of miRNAs in metal-induced beta cell dysfunction and potentially differentiate these from the mechanisms involved in obesity-associated T2D.

**2349** Manganese Transport and Toxicity in CRISPR-Cas9-Mediated SLCO3A10 Knock-Out Hep3b Cells

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Manganese (Mn), an essential metal, can be toxic at elevated levels. Mn toxicity can arise from occupational/environmental exposure, parenteral nutrition, hepatic conditions and certain genetic risks. Earlier this decade, such genetic risk was reported in the patients due to mutations in SLCO3A10, a Mn efflux transporter, leading to aberrant systemic Mn levels. Recent studies of genetically altered mouse model offered a better understanding of its importance in Mn homeostasis. To explore the function of SLCO3A10 in vitro, the current study employs a SLCO3A10 knockout Hep3b cell line (KO) designed using CRISPR-Cas9 gene editing tool. The mutations (in-deletion) leading to loss of function were confirmed by Sanger sequencing of target DNA. Nucleotide sequences showed a 5-BPs deletion and a T insertion in exon 1 of SLCO3A10 gene confirming the viable clone as a compound heterozygous (biallelic). qPCR analyses showed >70% reduction in SLCO3A10 expression of the KO engineered cells without any evident alteration in morphology or cell-growth pattern. Intracellular Mn concentrations measured using atomic absorption spectroscopy (AAS) indicated 1.7-fold and 3.6-fold (p<0.01) increases in KO cells (118 ±36 and 293 ±71 µM Mn/g protein) compared to WT cells (69 ±14 and 81 ±11 µM Mn/g protein) upon 24-hour exposure to 0.5 and 1 mM MnCl2, respectively. Cytotoxicity studies carried out using a series of concentrations of MnCl2 indicated the KO cells were susceptible to a short (24 hours) and a long-term (7 days) MnCl2 exposure when compared to wild-types. Such a difference may be attributed by intracellular Mn accumulation measured using AAS. Results from cytotoxicity studies carried out using series of concentration of ZnSO4 and CuSO4 showed a much smaller increase when compared to wild-type cells, suggesting the KO cells are susceptible to Mn toxicity, not to Zn and Cu. Transport studies carried out using radio-labeled Mn indicated a statistically significant reduction of 790% Mn import and export by KO cells when compared to wild-types. While the impaired Mn export was hypothesized, the impaired Mn import in these KO cells is currently under investigation. Such impaired import may result from suppression mutation/s leading to synthesis of a truncated inadequate Mn transporter. To address this, we generated a global Slc30a10 deficient (Slc30a10 KO/KO) mouse model using CRISPR/Cas9 to determine the localization of Slc30a10. Fluorescent images from Slc30a10 mice indicated Slc30a10 expression along the apical membrane of hepatocytes and enterocytes. Overall, our Slc30a10 KO/KO and Slc30a10 mice line suggest Slc30a10 is essential for early maintenance of Mn homeostasis.

**2535** Understanding the Role of the Manganese Transporter Slc30a10 in Developing Mice


Manganese (Mm) is an essential trace metal acquired through the diet. It is essential to regulate Mm levels as Mm can be toxic in excess. Mm excess leads to a Parkinson’s-like disorder which is often seen in industrial workers inhaling Mn-rich particles or fumes. Young individuals are believed to be particularly susceptible to the irreversible damages of Mn excess due to immature regulatory pathways of absorption and excretion. Therefore it is important to better understand early mechanisms of Mn metabolism to prevent Mn toxicity. In 2012, the first case of inherited Mn excess was identified. Patients exhibited systemic Mn excess, liver cirrhosis, a Parkinson’s-like disorder, and polycthemia (increased red blood cell counts). Mutations were identified in SLCO3A10, a protein previously thought to transport zinc. In this study we evaluate the potential role of SLCO3A10 in Mn homeostasis during development. We generate global Slc30a10-deficient (Slc30a10 KO/KO) mouse line. Slc30a10 KO/KO mice are smaller when compared to wildtype littermates, suggestive of early Mn toxicity. Metal analysis indicates postnatal day (PND) 14 Slc30a10 KO/KO mice exhibit comparable Mn levels when compared to Slc30a10+/- mice. However, by PND21 Slc30a10 KO/KO mice exhibit a 10-fold increase in brain Mn and a 2-fold increase in liver Mn levels. Interestingly Mn levels in Slc30a10 KO/KO mice decrease in the liver by PND28, suggesting the development of other Mn excretion pathways. Slc30a10 KO/KO mice also develop polycthemia between PND21 and PND28. Mn excretion studies also indicated PND21 Slc30a10 KO/KO mice excrete 40% less Mn than wildtype mice. In order to better understand the role of Slc30a10 in development, we also created an Slc30a10 W5F/W5F mouse line using CRISPR/Cas9 to determine the localization of Slc30a10. Fluorescent images from Slc30a10 W5F/W5F mice indicated Slc30a10 expression along the apical membrane of hepatocytes and enterocytes. Overall, our Slc30a10 KO/KO and Slc30a10 W5F/W5F mouse line suggest Slc30a10 is essential for early maintenance of Mn homeostasis.

**2352** Tungsten Enhances RANKL-Induced Differentiation of Osteoclasts in Bone

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Tungsten’s increasing presence in electronics, medical devices, and military applications position it as an emerging environmental toxicant, yet its potential health consequences are not well understood. As a contaminant in the environment, tungsten may become bioaccessible as tungstate, which can lead to exposure through water and soil. Like many metals, the highest concentration of tungsten accumulates in the bone, making the bone a long-term storage site and a source of chronic exposure. The bone is a dynamic organ that constantly undergoes 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 stem cells (MSCs). We report a global Slc30a10 deficient (Slc30a10 KO) mouse line using CRISPR/Cas9 to determine the localization of Slc30a10. Fluorescent images from Slc30a10 KO mice indicated Slc30a10 expression along the apical membrane of hepatocytes and enterocytes. Overall, our Slc30a10 KO and Slc30a10 W5F/W5F mouse line suggest Slc30a10 is essential for early maintenance of Mn homeostasis.
Tungsten is a ubiquitous metal that is used as an alloy in many different applications. Concerns about W toxicity have arisen after using tungsten in military ammunition instead of lead and depleted uranium. Tungsten is prevalent in commercial products due to its high density and malleability. Tungsten-nickel (W-Ni) is a developing alloy of interest in the aerospace industry due to its high resistance to corrosive environments. Other applications include orthopedic implants, magnetic heads for data storage, and military applications.

The harmful effects of tungsten toxicity have received considerable attention since the late 1990s. While the first studies limited to the analysis of toxicity in laboratory animals, the recent studies have focused on understanding the importance of tungsten in diseases related to cancer development. Tungsten toxicity has been shown to be a cause of cancer, and tungsten has been used as an ingredient in military ammunition instead of lead and depleted uranium. Tungsten is present in many commercial products due to its high density and malleability. Tungsten-nickel (W-Ni) is a developing alloy of interest in the aerospace industry due to its high resistance to corrosive environments. Other applications include orthopedic implants, magnetic heads for data storage, and military applications.

The harmful effects of tungsten toxicity have received considerable attention since the late 1990s. While the first studies limited to the analysis of toxicity in laboratory animals, the recent studies have focused on understanding the importance of tungsten in diseases related to cancer development. Tungsten toxicity has been shown to be a cause of cancer, and tungsten has been used as an ingredient in military ammunition instead of lead and depleted uranium. Tungsten is present in many commercial products due to its high density and malleability. Tungsten-nickel (W-Ni) is a developing alloy of interest in the aerospace industry due to its high resistance to corrosive environments. Other applications include orthopedic implants, magnetic heads for data storage, and military applications.
2357 Associations between Mercury Levels in Hair and Fish Consumption among Children in the South Area of Wakayama Prefecture, Japan

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People are mainly exposed to methylmercury (MeHg) through fish consumption. Evaluation of the relationship between mercury (Hg) levels in hair and fish consumption is important for risk assessment of MeHg exposure. Marine mammals such as whales and tunas are known to contain high concentrations of Hg. In the south area of Wakayama Prefecture, Taiji residents traditionally eat whale and Nachikatsuura residents frequently eat tuna compared with other areas. Therefore, some of these residents may be exposed to higher levels of Hg. Assessments of MeHg exposure through fish consumption have been conducted for adult residents in this area, but no studies have estimated MeHg exposure in children who are highly susceptible to MeHg. The present study examined the associations (Spearman’s rank correlation coefficients) between Hg levels in hair in 106 children (Taiji residents: 29; Nachikatsuura residents: 77) at 7 years of age and their fish consumption, including marine mammals, using a food frequency questionnaire (FFQ) and food model for assessing MeHg exposure. The Hg levels in the hair of all 106 study subjects had a median of 2.7 µg/g, maximum of 21.3 µg/g, and minimum of 0.6 µg/g. The correlations between Hg levels in hair and consumption of whale, tuna, and 12 other fish species among the 29 subjects in Taiji were whale (r=0.71), tuna (r=0.21), and 12 other fish species (r=0.27). The correlations between Hg levels in hair and consumption of whale, tuna, and 12 other fish species among the 77 subjects in Nachikatsuura were whale (r=0.17), tuna (r=0.36), and 12 other fish species (r=0.09). The correlations between Hg levels in hair and consumption of tuna and 12 other fish species in the subjects who did not eat whale were tuna (r=0.39) and 12 other fish species (r=0.17). These findings indicate significant positive correlations between Hg levels in hair and areas with specific eating habits such as consumption of whale in Taiji or consumption of tuna in Nachikatsuura. The FFQ with food model used in this study and resulting information will be useful for future assessments of the health risks of MeHg exposure through fish consumption in children.

2358 Methylmercury Exposure Impacts Cell-Cycle Progression of Myoblasts


Methylmercury (MeHg) is a pervasive environmental toxicant, well-established to affect the nervous system. Recent evidence, however, has identified muscle as a novel target of MeHg toxicity. This is an attractive target owing to the characteristic muscle weakness observed in MeHg intoxicated humans. There is minimal evidence, however, on the direct effect of MeHg on muscle development. A previous study demonstrated MeHg decreases myogenin (MyoG) expression in C2C12 myoblasts induced to differentiate. MyoG is a transcription factor, which is normally upregulated within 24 hours (hr) of myoblast differentiation induction. It promotes expression of several factors important to myoblast muscle development. Here, we aim to explore the potential mechanism by which MeHg alters normal differentiation of myoblasts. It is our hypothesis MeHg perturbs myoblast differentiation, and maintains the undifferentiated state even under differentiation conditions. For our experiments, we also used C2C12 myoblasts. This in vitro model allows straightforward differentiation of myoblasts, immature, single-nucleated muscle cells, to myotubes, mature, multi-nucleated muscle fibers. We treated C2C12 with MeHg (0.5 or 2.5 µM) in growth media (myoblasts continue to proliferate) for 24 hr, then switched to low serum media (myoblasts induced to differentiate) without MeHg for another 24 hr. RNA and protein were isolated post-exposure for quantitative PCR and whole-cell western blot, respectively. We detected a significant decrease in MyoG expression at 2.5 µM, which translated to decreased MyoG protein as well at 0.5 and 2.5 µM. To elucidate further the effects of MeHg on cell-cycle, we examined p21, a cyclin-dependent kinase that is upregulated in myoblasts to signal terminal differentiation. We observed a significant decrease in p21 protein at both 0.5 and 2.5 µM. Moreover, we detected a persistent undifferentiated state; MyoG is downregulated and unable to promote muscle development, and p21 decreases, impacting terminal differentiation signals. It is necessary, however, to assess relative proportion of myoblasts to myotubes, as well as cell proliferative capacity under this culture condition, to confirm maintenance of the myoblast state. Supported by NIEHS R01ES025721 and T32ES007026.

2359 Tissue-Specific Role of Nrf2 (Nrf2) in Methylmercury-Induced Toxicity during Drosophila melanogaster Metamorphosis

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Methylmercury (MeHg) is a ubiquitous environmental toxicant known to target the developing nervous system. However, recent evidence implicates that MeHg also targets developing muscles, which may explain motor deficits seen in developmentally exposed children. Previous work in Drosophila melanogaster has indicated that developmental exposure to MeHg can inhibit eclosion, a stereotypic muscle-dependent behavior required for emergence of the adult fly from the pupal case. Normal eclosion behavior is thought to require the dorsal internal oblique muscles (DIOMs), a larval-derived muscle group that persists through metamorphosis. Thus, we hypothesized that perturbation of these muscles by MeHg could underlie the failure to eclose. MeHg can elicit toxicity at the cellular level by reacting with sulfhydryl groups on proteins or via oxidative stress induction. Moreover, MeHg has been shown to activate the Nfr2 antioxidant response pathway. Nfr2 is a transcription factor which, under basal conditions, is constitutively degraded. Exposure to MeHg prompts Nfr2 activation, and promotes expression of anti-oxidant genes. The Nfr2 pathway has primarily been studied in regard to xenobiotic defense, aging, and cancer in mature organisms. Less is known about the developmental role of Nfr2. Using the Drosophila model, we investigated the impact of developmental MeHg exposure on CncC, the Nfr2 homolog, in function in developing muscle. We hypothesized that CncC protects developing muscles from MeHg toxicity via enhanced transcription of antioxidant genes. To validate this, we show that the Nfr2 pathway is activated in Drosophila after exposure to MeHg in both adults and pupa by qPCR and Western blot. Further, we observe MeHg induces a collapsed myosporid phenotype, observed by fluorescence microscopy, in the DIOMs consistent with a parallel decrease in eclosion ability. Attempts to rescue eclosion with targeted overexpression of CncC reveals unexpected and distinct activity in muscles and neuro-muscular connective tissue, whereby overexpression in muscles was highly deleterious and lethal, and overexpression in neurons successfully rescued eclosion ability after developmental exposure to MeHg. Interestingly, CncC is seen to be endogenously expressed in developing DIOMs, which may be required for the proper function of the muscles in development and metamorphosis. Our data demonstrates a potential tissue specific activity for Nfr2 signaling during development that can directly differential toxic effects of MeHg. Supported by RO1ES025721 and T32ES007026.
the worms fed the high fat diets. Taken together, our data suggest a diet by
toxicant interaction in *C. elegans*, where dietary fat levels directly influence
the nematode’s response to MeHg exposure.

### 2361 Disposition and Toxicity of Cyanide-Mercury Complexes in Target Organs

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Mercury (Hg) is an environmental toxicant that is prevalent throughout the
world. Humans may be exposed Hg via inhalation, consumption of contami-
nated food, and/or exposure to Hg-containing substances. A major source of
environmental Hg is artisanal and small-scale gold mining (ASGM). Hg is
used to extract gold from ore; however, this process is very inefficient and a
large percentage of gold remains in the tailings. Cyanidation is used to extract
additional gold from processed ores. Because these ores are contaminated with
Hg from initial extraction attempts, there is significant concern that Hg
interacts with cyanide (CN) to form a Hg-CN complex. Humans may be ex-
posed to Hg-CN complexes via the ingestion of contaminated fish, ingestion of
fruits and vegetables irrigated with contaminated water, and ingestion of
shrimp from farms utilizing contaminated water. The purpose of the current
study was to determine where Hg-CN complexes accumulate in mammalian
organs/tissues and to determine the toxicity associated with exposure to
these complexes. Rats were injected with mercury(I)cyanide (0.1, 0.2, 0.3, and
0.4 mg/kg), containing radioactive mercury. The accumulation of Hg-CN was
measured in blood, kidney, liver, spleen, brain, urine, and feces. Histological
analyses of kidney and liver revealed injury even at lower doses. Immune re-
sponses in the spleen were suppressed by exposure to Hg-CN. Exposure to
Hg-CN has significant toxicological effects on mammalian systems and thus,
the presence of Hg-CN complexes in the environment is a significant human
health problem.

### 2362 Suppression of E2F1 and RAD51 in Chromate-Induced Failure of Homologous Recombination

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Lung cancer is a leading cause of cancer death, however while this disease is
largely attributed to smoking approximately 1 in 5 people with lung can-
cer never smoked. The mechanisms of how lung carcinogens cause cancer
are poorly understood. Studies show that chromosome instability is an early
event in lung cancer and contributes to this end point. Many metals including
hexavalent chromium (Cr(VI)) are known to induce chromosome instability.
Additionally, we have shown particulate Cr(V) causes DNA double strand
breaks and prolonged exposure impairs the effector step of the high fidelity
repair pathway, homologous recombination. The homologous recombin-
ation pathway is crucial in preserving genomic stability and preventing car-
cinogenesis. Data in our human lung cell model show longer exposures to
particulate Cr(VI) lead to loss of the critical effector step in the homologous
recombination pathway through impaired RAD51 function while preceding
steps remain functional. It is currently unknown how Cr(VI) impacts RAD51
function. The transcription factor E2F1 has previously been shown to be in-
olved in homologous recombination through transcription of DNA repair
proteins and also plays a role in the homologous recombination response to
DSBs. The objective of this study was to show prolonged exposure to par-
ticulate Cr(VI) inhibits the RAD51 response through E2F1 affecting mRNA ex-
pression and protein levels and localization. We found exposure to particulate
Cr(VI) reduced RAD51 and E2F1 mRNA and protein levels at all exposures. We
also observed alteration in protein half-life, but inhibited protein focus
formation in response to prolonged particulate Cr(VI) exposure. These results
provide mechanistic insight into the carcinogenesis of particulate Cr(VI) in
human lung cells and indicate a role of the transcription factor E2F1 in the loss
of the homologous recombination response. These data further build a foun-
dation to directly investigate how E2F1 is modulating the RAD51 response to
particulate Cr(VI) exposure. This work was supported by the National Institute
of Environmental Health Sciences [ES016893 to J.P.W] and the Jewish Heritage
Foundation for Excellence.

### 2363 Homologous Recombination Repair Protects against Genomic Instability in Bowhead
Whale Lung Cells after Prolonged Particulate Chromate Exposure

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Particulate hexavalent chromium (Cr(VI)) is a well-established human lung
carcinogen, however its carcinogenic mechanism is poorly understood. Cr(VI)
induces DNA double strand breaks leading to genomic instability and carcino-
genesis in human lung cells. Homologous recombination (HR) repair protects
against genomic instability by maintaining high genomic fidelity during the
repair of DNA double strand breaks. Prolonged particulate Cr(VI) suppresses
HR repair while simultaneously inducing DNA double strand breaks in human
lung cells. Whales are long lived species living in a complex environment that
puts them at risk for long-term exposure and accumulation of environmental
contaminants. However, they have low cancer rates, which may be due to
better DNA protective mechanisms. How whales evade carcinogenesis is
unknown. This study focuses on the effect of particulate Cr(VI) exposure in
whale lung cells to determine if they exhibit protective mechanisms against
Cr(VI)-induced chromosome instability. Our study shows zinc chromate in-
duces concentration-dependent increases in cytotoxicity, chromosome dam-
age and DNA double strand breaks after both acute (24 h) and prolonged (120
h) exposure in whale lung cells. In response to zinc chromate-induced breaks,
HR was increased after both acute (24 h) and prolonged (120 h) zinc chromate
exposure. Our results indicate the response to prolonged Cr(VI)-induced cyto-
xicity, genotoxicity and HR repair activity are different between whale and
human lung cells. Future investigation of the differences in how human and
whale cells respond to chemical carcinogens may provide valuable insight
into mechanisms of preventing chemical carcinogenesis. The work was sup-
ported by the National Institute of Environmental Health Sciences [ES016893 to
J.P.W].

### 2364 Do Dysregulated Cellular Energetics Play a Role in Hexavalent Chromium-Induced
Human Lung Carcinogenesis?

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Hexavalent chromium, Cr(VI), is a known human carcinogen that is a world-
wide environmental health concern. It is established that reactive oxygen
species, genomic instability, and DNA damage repair deficiency are import-
ant contributors to Cr(VI)-induced carcinogenesis mechanism. Some cancer
hallmarks remain understudied for the mechanism of Cr(VI)-induced carcino-
genesis. Increased de novo lipogenesis and the ‘Warburg Effect’ are an im-
portant in the mechanism of carcinogenesis and tumorigenesis in multiple
types of cancer. We recently reported that Mitochondrial respiratory dys-
function occurs after tumor development and not during the initial Cr(VI)-
induced carcinogenesis. Continuing this work we investigated the changes in
lipid metabolism and the ‘Warburg Effect’ in Cr(VI)-transformed human lung
cells. We used three human lung cell lines (human lung epithelial cells (BEAS-2B
and BEP2D cells) and human lung fibroblasts (WTHBF-6 cells)). Our results
showed an increased lipogenesis (palmitic acid levels) and Cr(VI) and
increased expression of lipogenesis proteins [ATP citrate lyase (ACLY), and
fatty acid synthase (FASN) expressions]. Using a drug (C75) for inhibition of
FASN, we found that C75 treated Cr(VI)-transformed cells had decreased
proliferation and loss of colony formation in soft agar. Interestingly, we also
found that Cr(VI)-transformed cells had no major changes in their glycolytic
function as measured by the Seahorse Analyzer when compared to their pas-
sage matched control cells and there were no observed increases in Cr(VI)-
transformed cells’ extracellular L-lactate levels. In conclusion, these data show
that Cr(VI)-transformation in vitro caused increases in lipid metabolism and
this increase is important for Cr(VI)-transformed cell cancer properties. In con-
trast, Cr(VI)-transformed cells did not exhibit increased anaerobic glycolysis
(‘Warburg Effect’). Therefore, future work is aimed at elucidating the mecha-
nism that is increasing lipogenesis and not increasing anaerobic glycolysis in
Cr(VI)-transformed human lung epithelial cells and lung fibroblasts.
Hexavalent chromium (Cr(VI)) compounds are known human lung carcinogens; but the carcinogenic mechanism is poorly understood. Cr(VI) induces DNA damage which normally leads to apoptotic responses to avoid transformation and carcinogenesis. Evasion of apoptosis is a hallmark of carcinogenesis, but it is unknown how Cr(VI)-damaged cells are able to escape cell death. We exposed human lung cells to low concentrations of zinc chorate for 6 months. During treatment we observed cell death using a colony forming assay. We also monitored changes in chromosome number and structure. In the middle, and at the end of treatment, cells were tested for anchorage independence. We found Cr(VI) induced cell death during the first 25 days of exposure with lower concentrations (0.0125 and 0.025 µg/cm²) while the highest concentration, 0.05 µg/cm², caused significant cell death initially, followed by a period of delayed 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 with the high treatment. Additionally, cells that escaped particular Cr(VI)-induced cell death exhibited significant amounts of structural and numerical changes. Control cells at all time points showed normal karyotypes. For treated cells, on day 5, 24, 26, and 30 percent of cells had abnormal karyotypes at 0.0125, 0.025 and 0.05 µg/cm² zinc chorate, respectively. On day 70, 46, 52, and 48 percent of cells had abnormal karyotypes at the same concentrations. On day 180, 18, 72, and 84 percent of cells had abnormal karyotypes at the same concentrations. Treatment-induced aneuploidy were observed at early time points compared to structural changes. None of the mass treated cells grew colonies in soft agar, however, clonal cell lines developed after 180 days of treatment indicated that 30% exhibited neoplastic transformation with a high degree of numerical and structural chromosome instability. These data support a hypothesis that Cr(VI)-treated cells can evade apoptosis and transform into chromosomally unstable cells that continue to survive and grow. This work was supported by NIHES grant ES016893 (J.P.W.) and the University of Louisville School of Medicine Basic Grant Program (S.S.W.).

- **The Fate of Cells That Escape Cr(VI)-Induced Cell Death**
- **Hexavalent Chromium Disrupts Chromatin Architecture**
- **The Effect of GMDC in Overcoming Cisplatin-Induced Systemic Toxicity**
- **Leaving Ligand Effects on Cytotoxicity, Solubility, and Reactivity of Monofunctional Platinum(II) Complexes**

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**References:**

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Hexavalent chromium compounds are well-established respiratory carcinogens used in industrial processes. While inhalation exposure constitutes an occupational risk affecting mostly chromium workers, environmental exposure from drinking water or a widespread survival at day 70 of treatment, affecting millions of people throughout the world. Cr(VI) is genotoxic, forming protein-Cr-DNA adducts and silencing tumor suppressor genes, but its mechanism of action at the molecular level is poorly understood. Our prior work using FAIRE showed that Cr(VI) disrupted the binding of transcription factors CTCF and AP-1 to their cognate chromatin sites. Here, we used two complementary approaches to test the hypothesis that chromium perturbs chromatin organization and dynamics. DAmPOS2 analyses of MNase-seq data identified several chromatin alterations induced by Cr(VI) affecting nucleosome architecture, including occupancy changes at specific genome locations; position shifts of 10 nucleotides or more; and changes in position amplitude or fuzziness. ATAC-seq analysis revealed that Cr(VI) disrupted the accessibility of chromatin regions enriched for CTCF and AP-1 binding motifs, with a significant co-occurrence of binding sites for both factors in the same region. Cr(VI)-enriched CTCF sites were confirmed by ChIPseq and found to correlate with evolutionarily conserved sites occupied by CTFC in vivo, as determined by comparison with ENCODE-validated CTFC datasets from mouse liver. In addition, more than 30% of the Cr(VI)-enriched CTFC sites were located in promoters of genes differentially expressed from chromium treatment. We propose that Cr(VI) disrupts 3-dimensional chromatin organization and boundary formation between topologically associated domains in chromosomes, destroying the interactions between transcription regulatory sequences. Our results support the conclusion that Cr(VI) exposure promotes broad changes in chromatin accessibility and suggest that the subsequent effects on transcription regulation may result from disruption of CTFC binding and nucleosome spacing, implicating transcription regulatory mechanisms as primary Cr(VI) targets. Supported by NIERS R01 ES010907.

**2368 The Effect of GMDC in Overcoming Cisplatin-Induced Systemic Toxicity**


Cisplatin is a platinum-based chemotherapeutic drug widely used in the treatment of various cancers such as testicular, ovarian, lung, bladder, and cervical cancers. However, its use and the dosage range applied have been limited by severe side effects (e.g., nephrotoxicity and ototoxicity) and by the acquired resistance to cisplatin of patients during treatment. Metal-chelating agents have shown promising potentials in overcoming these problems associated with platinum drugs. We developed a new chelating chemical, sodium (S) 2, (dithiocarbamylato) (2S,3R,4R,5R)-2,3,4,5,6 pentahydroxyhexyl) (aminol) - and (dithiocarbamylato) butanoate (GMDC). In this study, we examined the effects of GMDC in modifying the cisplatin-caused systemic adverse effects in vitro and in vivo. GMDC exposure showed no cytotoxic effects on normal human kidney HK2 cells following 24h exposure at levels up to 1000 µM, and can dramatically reduce cisplatin-induced apoptosis and ototoxicity in HK2 cells. Results also showed a decreased amount of platinum in cells co-treated with GMDC in cisplatin rescue experiments. In 4T1 breast cancer xenograft mice model, it was shown that GMDC reduced cisplatin-induced nephrotoxicity by reducing cisplatin deposition in the kidney and attenuating cisplatin-induced elevations in BUN and plasma CRE. GMDC also ameliorated renal tubular dilation and vacuolation, and necrosis of glomeruli and renal tubule cells. Auditory brainstem response (ABR) test showed that cisplatin treatment decreased the hearing sensitivity of mice at all frequencies of stimulus, but GMDC reduced the hearing sensitivity loss, therefore alleviating the ototoxicity caused by cisplatin. Furthermore, cisplatin-induced other systemic toxicities were improved by GMDC as supported by the increased body weight and reduced hematotoxicity and hepatotoxicity. Importantly, co-treatment of cisplatin with GMDC does not affect cisplatin’s antitumor efficacy. The tumor growth, size, and metastasis were all comparable between cisplatin only treatment group and cisplatin and GMDC co-treatment group. In conclusion, our study suggests that GMDC reduces cisplatin-induced toxicity by preventing the accumulation and as well as the reduction of intracellular cisplatin, without compromising cisplatin therapeutic activity, supporting the further development of GMDC as a clinical agent in overcoming cisplatin’s toxicity and resistance.

**2369 Leaving Ligand Effects on Cytotoxicity, Solubility, and Reactivity of Monofunctional Platinum(II) Complexes**

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Monofunctional platinum(II) complexes, such as phenanthroplatin and pyriplatin, have notably different characteristics from the bifunctional anticancer complexes, such as cisplatin and oxaliplatin. The traditional bifunctional complexes covalently bind to DNA and predominantly form a 1,2-GG-intrastrand crosslink which leads to DNA distortion, transcriptional inhibition, and ultimately apoptosis. The monofunctional complexes bind to DNA at a single
guanine residue, without causing the DNA helix to distort, resulting in RNA polymerase II transcribing complex stalling at the platinum-DNA adduct and inhibition of transcription. This difference in DNA binding leads to different mechanisms of transcription inhibition, which correlates to cytotoxic effect. Monofunctional platinum(II) complexes have unique properties that may be exploited to target cancer cells without producing the toxic side effects associated with the currently approved platinum anticancer drugs. To advance the understanding of these monofunctional platinum(II) complexes, this study replaced the chloride leaving ligand with an acetate group, which should increase solubility and alter the rate of reactivity with key amino acid and nucleotide targets. Phenanthriplatin and pyriplatin compounds were reacted with silver acetate to form insoluble silver chloride and the desired compound. Proton nuclear magnetic resonance (1H NMR) spectroscopy was utilized to characterize the new compounds and conduct kinetic assays with guanosine 5′-monophosphate (5′-GMP). Rate constants of 2.9 x 10^{-4} M^{-1} s^{-1} and 1.6 x 10^{-5} M^{-1} s^{-1} were determined for pyriplatin and phenanthriplatin with 5′-GMP, respectively. A preliminary rate constant of 7.0 x 10^{-6} M^{-1} s^{-1} was determined for the newly synthesized pyriplatin-acetate complex. Ligand exchange kinetics directly influences the anticancer activity and toxicity of platinum drugs. Initial results indicate that the solubility is increased, and the rate of reaction is decreased by the acetate ligand. Further studies are currently being conducted to determine the cytotoxic effect of these novel platinum(II) compounds in mammalian cellular models (HEK293 and NTERA-2).

2370 The Effectiveness of a Monofunctional, Novel Platinum (II) Compound on Mammalian Cell Viability

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For decades, platinum containing compounds have been used for the treatment of different types of cancer. In the United States, there are currently three different platinum compounds approved by the US FDA for use in the treatment of cancer: cisplatin, carboplatin, and oxaliplatin, all bifunctional compounds. These bifunctional compounds have two leaving groups, thus attached at two sites on the DNA molecule inducing interstrand cross-links and distorting the DNA. Phenanthriplatin, a cisplatin derivative, is one of several platinum compounds currently being considered as a treatment for cancer. Unlike cisplatin, phenanthriplatin is monofunctional, having only one leaving group to attach to the DNA without distortion the macromolecule. Bifunctional and monofunctional platinum(II) complexes bind to DNA by different mechanisms but ultimately lead to cell death. Here, Chloro-[2-(4-methyl-1,4-diazepan-1-yl)ethanamine] platinum(II) chloride (compound 1), a platinum compound with a similar steric profile to phenanthriplatin was reacted with both nucleotide, 5′-Guanosine monophosphate (5′-GMP), and the amino acid, N-Acetyl-L-methionine (N-AcMet). In the competition, N-AcMet products were formed from 24 hours, but 5′-GMP remained unreacted until several days. However, with time, 5′-GMP can replace N-AcMet. Thus, compound 1 shows a more traditional small molecule reactivity than other sterically hindered trinuclear compounds studied in the lab. Current studies have explored the cytotoxicity the compound in a cellular model of prostate cancer (NTERA-2) and control cells (HEK293). Current MTT data suggests that this compound, unlike phenanthriplatin, does not have higher toxicity than cisplatin.

2371 Development and Validation of an Analytical Method for Total Thallium in Rodent Plasma and Tissues by ICP-MS


Thallium (Tl) is a naturally occurring element and exists in +1 or +3 state. Thallium (Tl) is a heavy metal that naturally occurs in the earth's crust. Thallium has been used as a poising agent and induces a spectrum of adverse health effects, including nervous system and neurotoxicity. Chronic human exposure to thallium compounds occurs due to its presence as a contaminant in drinking water. Currently, there is insufficient toxicological data to support derivation of a human reference dose for thallium compounds. To address this data-gap, the National Toxicology Program (NTP) performed both perinatal rat range-finding and 14-day adult mouse toxicity studies of thallium (I) sulfate. Time-mated SD rats (n=12-20 per group) and offspring were exposed from gestational day 6 (GD 6) to postnatal day 28 (PND 28) via dosed drinking water containing 0, 3.13, 6.25, 12.5, 25, or 50 mg/L thallium (I) sulfate. All 25 and 50 mg/L groups were removed from study by PND 0 due to morbidity observations. Thallium exposure concentrations ≤ 2.5 mg/L did not impact dam fecundity or offspring survivability (PND 4-28); however, whole-body alopecia was observed in all offspring exposed to 12.5 mg/L thallium. Exposure to 12.5 mg/L also resulted in lower PND 28 bodyweights in males (-12%) and female (-9%) offspring, relative to controls. Total thallium concentration in maternal plasma, amniotic fluid (GD 18), fetal hematocrit (GD 18), and pup plasma (PND 4) provide evidence of placental and fetal transfer of thallium. Adult B6C3F1 mice (n=5 per sex/group) were exposed for 14 days via dosed drinking water containing 0, 6.25, 12.5, 25, 50, or 100 mg/L thallium (I) sulfate. Observed mortality led to the removal of the 100 mg/L group by study day 9. At study conclusion, mice exposed to 50 mg/L and > 100 mg/L displayed 20% (5/25) and 15.2% (3/19) (males) and 23.3% (11/47) (females) lower bodyweights relative to controls, respectively. Plasma thallium concentrations ranged from 0.3-2.734 ng/mL in male mice and 0.1-0.1998 ng/mL in female mice. Evidence of thallium toxicity was observed in two rodent species following subchronic drinking water exposure; these results will inform future studies to further characterize thallium hazard and safeguard public health.
production and the activation of JNK. Pretreatment with the antioxidant NAC and SP600125 (JNK inhibitor) effectively reversed Sb−−−induced β-cell apoptosis and related signals. In conclusion, these results suggest that Sb exerts its cytotoxicity on pancreatic β-cells by inducing the apoptosis through ROS generation-induced JNK activation downstream-triggered apoptosis pathway and provide further evidence to confirm the possible role of Sb as an environmental risk factor for diabetes.

3-Month Toxicity Studies of Tetravalent and Pentavalent Vanadium Compounds in Hsd:Sprague-Dawley SD Rats and B6C3F1/N Rice via Drinking Water Exposure

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The National Toxicology Program performed 3-month toxicity studies of tetravalent (vanadyl sulfate; VS) and pentavalent (sodium metavanadate; SM) vanadium compounds in drinking water, due to potential human exposure and lack of robust toxicity data. Time-mated Hsd:Sprague Dawley SD rats were exposed via dosed drinking water during gestation (beginning GD6) and lactation. Pups were exposed in utero, during lactation, and continued exposure for 3 months post-weaning. Adult B6C3F1/N mice were exposed for 3 months. Animals were exposed to VS at 0, 21, 41.9, 83.8, 167.5, or 335 mg/L, or to SM at 0, 3.13, 6.25, 12.5, 250, or 500 mg/L. There was higher morbidity in the 500 mg/L SM dams and pups throughout the postnatal period. There were lower percent live pups at birth (-25%), lower number of viable pups at PND4 (-45%; pre-standardization) and throughout lactation after litter standardization (-28%; PND4-28) compared to controls. There were no effects on littering or pup survival in VS exposed dams or pups. For both compounds, in rats and mice, water consumption (g/day) was lower in male and female pups exposed to SM, compared to controls. In male and female mice exposed to SM, there were consistent increases in erythrocytes and reticulocytes and decreases in hematocrit and hemoglobin; changes in rats and in VS exposed animals were sporadic. In general, organ weight effects were attributed to body weight increases, except for treatment-related decreases in thymus weights for SM mice. Histopathological findings were limited for both sex/species, exposed to either compound. Based on plasma and urine total vanadium levels, and estimated vanadium consumption from water consumption data, the exposure to vanadium from SM appears to be higher than from VS. For both SM and VS, internal exposure increased proportionally with increasing estimated vanadium consumption. In general, effects were more frequently observed in SM exposed animals which may be attributable to higher systemic exposure to vanadium at similar compound concentrations.

Application and Safety Concern of Bismuth Materials and the Role of Autophagy in Bismuth Induced Nephrotoxicity Associated Mechanisms

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Bismuth is widely used in metallurgy, cosmetics industry and medical diagnostics and recently, bismuth nanoparticles (BiNP) have been made and proved to be excellent CT imaging agents. We have synthesized bovine serum albumin based BiNP for CT imaging but we find a temporary kidney injury by BiNP. Due to the previous reported risk of bismuth on human health, we extended our studies on the mechanisms for BiNP induced nephrotoxicity. First, we found that BiNP can induce autophagy in human embryonic kidney cells, shown by the increase of monodansylcadaverine fluorescence staining and the amount of LC3II that can be inhibited by 3-MA. BiNP were capable of entering cells in a dose and time dependent manner by fluorescence, transmission electron microscopy and element detection methods. Blood biochemical analysis for in vivo studies indicated (g/day) was lower in control and blood nitrogen associated with cell apoptosis/necrosis and intraluminal cast formation in proximal convoluted tubules, indicating BiNP induced acute kidney injury (AKI) in mice. During AKI, we found an increase in LC3II, while the autophagic flux indicator p62 remained unchanged. Chloroquine and rapamycin was used to activate autophagy in AKI caused by BiNP. The results showed that BiNP induced AKI was significantly attenuated when autophagy was further promoted by rapamycin and AKI became severe when chloroquine was applied. In vitro studies also indicated BiNP induced autophagy, evident in autophagic vacuole formation, and increased level of autophagy related proteins including LC3II, Beclin1 and Atg12. Specifically, reactive oxygen species (ROS) generated by BiNP could be the major inducer of autophagy, because the ROS blocking attenuated autophagy. Autophagy induced by BiNP was primarily regulated by AMPK/mTOR signal pathway and partially regulated by Akt/mTOR. We also extended our findings by selecting five bismuth compounds and the results showed that bismuth nitrate, bismuth oxychloride, bismuth citrate, colloidal bismuth subcitrate and bismuth nanomaterials all induced slight cytotoxicity accompanied with autophagy. Our study provides fundamental theory to bismuth induced toxicity and the safety concern of bismuth metal needs to be emphasized.

Pulmonary Function in Veterans Exposed to Depleted Uranium

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In 1991, a group of Gulf War I (GWI) Veterans was exposed to depleted uranium (DU) during friendly fire incidents. A cohort of them underwent biennial surveillance at the Baltimore VA for potential DU-related health effects. The primary exposure in this cohort was via inhalation, but a subset have retained fragments and persistently elevated uranium urine (U) levels suggesting ongoing systemic exposure. Potential airways toxicity from exposure to DU oxides, particulates, and blast impact have prompted respiratory health monitoring, although no U-specific differences have been identified. We continue to follow this cohort with the hypothesis that those with higher DU body burdens may display a higher frequency of pulmonary abnormalities as measured by impulse oscillometry (IO) and spirometry when compared to those with lower DU levels. Between April and June of 2017, 42 of a dynamic cohort of 80 DU-exposed GWI Veterans were evaluated during a 3-day visit. Medical history and exposure questionnaires, 24-hour urine U (uU) collections, IOs, and spirometry testing were obtained. The mean percent of spirometry and uU values were compared between Veterans with high vs low uU values for forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), and the ratio of FEV1/FVC and airways resistance at 5 Hz (R5). Overall, mean spirometry and IOs values fell within the normal range. On spirometry, the mean percent predicted values for flow (low lung volume): FEV1, 90.9% vs 86.1%; FVC, 93.4% vs 91.2%. The mean FEV1/FVC ratios for both groups were above the 70% threshold for obstructive abnormality and were similar at 76.4% (high) and 73.5% (low). On IOs testing, the mean percent predicted values for total airways resistance (R5) were below the threshold for obstruction of 150% (112% high U, 123% low U). Higher DU body burden was not associated with worsening spirometric or lung function impaired by spirometry or IOs testing. Mean values obtained on both tests indicate that lung function remains normal in the cohort overall, now 26 years after the initial insult and without evidence of effect from the U burden.

Minimal Uranium Immunotoxicity following a 60-Day Drinking Water Exposure to Uranyl Acetate in Male and Female C57BL/6J Mice


High levels of uranium exist in air, water, and soil in the Navajo Nation, due in part to ~500 abandoned uranium mines located in the rural southwestern United States. In a recent report evaluating metal concentrations in unregulated water sources within the Navajo Nation, 75% had detectable levels of uranium, with 12.5% having levels above the regulated limit of 30 ppb. In addition, uranium was found to co-occur with other metals, including arsenic, which could magnify negative health effects in this population. Human exposures in the Navajo Nation are associated with increased prevalence of metal-associated diseases, including some evidence of immune dysfunction. However, little is known about the accumulation of uranium in immune organs or the immunotoxicity of uranium. We recently published, that oral exposure to uranium, in the form of uranyl acetate, results in overall low tissue retention of uranium (<0.01%), with very little accumulation in immune organs (blood, bone marrow, spleen, and thymus) of male and female mice. In the present study, we characterized the immunotoxicity of uranium, in the form of uranyl acetate (UA), following a 60-day drinking water exposure to 5 and 50 ppm in male and female C57BL/6J mice. The following immunotoxicity endpoints were evaluated: hematology, immunophenotyping of the bone marrow, spleen, and thymus, and immune cell function (lymphocyte mitogenesis and T-dependent antibody response). Uranium exposure had subtle impacts on the immune endpoints evaluated, likely as a result of low uranium accumulation at these sites. The only significant immunological alterations were a slight decrease in the numbers of splenic NK T-cells and macrophages of exposed male mice. Future work will focus on the immunotoxicity of uranium and arsenic co-exposures as well as on how changes in dietary conditions, such as supplemental iron, may affect uranium immune tissue.
Recent reports have demonstrated the association between heavy metal exposure and preterm birth. Preterm births are classified as early and late, depending on the duration of pregnancy, and are associated with increased risk of congenital illnesses such as heart failure, asthma, etc. Particularly, early preterm births carry a higher risk of mortality; however, the differential effects of heavy metal exposure on early and late preterm births are unknown. Objectives: To analyze the association between maternal whole blood concentrations of heavy metals, such as cadmium (Cd), lead (Pb), mercury (Hg), selenium (Se), and manganese (Mn) that are common toxicants in Japan, and early and late preterm births. The data of 14,847 pregnant women who were participants of the Japan Environment and Children’s Study were used. Data of the self-questionnaire pertaining to the first trimester (T1), second/third trimester (T2), and medical records after delivery were analyzed. Women were divided into two groups: early preterm (22 to < 34 weeks) and late preterm (34 to < 37 weeks). Maternal blood samples for measuring heavy metal concentrations were collected in T2 (pregnancy weeks: 14-39). The participants were classified into four quartiles (Q1-Q4) according to increasing heavy metal levels. The rate of preterm birth was 4.5%. After controlling for confounding factors such as age, pre-pregnancy body mass index, smoking, partner’s smoking, drinking habits, gravidity, parity, number of cesarean deliveries, uterine infections, household income, educational levels, and sex of infant, Cd levels were found, by multivariable logistic regression analysis, to be significantly associated with early preterm birth (p = 0.002), with odds ratio for early preterm birth of 1.91 (95% confidence interval: 1.12-3.27, P = 0.018) in subjects of Q4 compared with in subjects with term birth (≥ 37 weeks). Maternal blood Cd levels during pregnancy are positively associated with the risk of early preterm birth among Japanese women. Identification of the main source of maternal Cd exposure may contribute to the prevention of early preterm births and health maintenance of mothers and their infants in the future.

Effects of Model, Method of Collection, and Topography on Chemical Elements and Metals in the Aerosol of Tank-Style Electronic Cigarettes

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Over the past decade, electronic cigarettes (EC) have evolved from the classic 3-piece model to a cartomizer style, to a disposable unit, and finally to the tank style. These modified EC come in a variety of styles and shapes consisting of larger, more powerful batteries, replaceable atomizing units, and large volume tanks. The purpose of this study was to examine the effect of puffing topography and method of collection on 19 elements/metals in aero.

Improvement of Organotypic Slice Culture of Mouse Cerebral Cortex to Evaluate M1-Type Microglial Activation

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Microglia (M0) at resting state sense microscopic changes in brain environments and activate to neurotoxic microglia (M1), which release inflammatory cytokines that are involved in neuronal cell death. It has been focused that M1-activation in cerebral cortex is implicated in various neurodegenerative diseases. Although organotypic slice culture of hippocampus of rats is widely used to study the crosstalk between microglia and neurons, little is known about the properties of microglia in the slices of cerebral cortex from mice with high applicability of the transgenic techniques. In this study, we aimed to establish a modified method for organotypic slice culture of mouse cerebral cortex that can accurately evaluates M1-activation. A few groups reported that cerebral cortices of embryonic day (E) 14 - postnatal day (P) 3 mice are available for organotypic slice culture, however microglia undergo proliferation and migrate to the cerebral cortex at P7 - P15, acquiring their mature phenotype. Thus, there is a possibility that the M1 phenotype cannot be evaluated correctly in slices prepared by conventional method, since the microglia are immature. To confirm the point, we examined the effect of postnatal day on the M1-activation by lipopolysaccharide (LPS), a classic inducer of the M1-activation. We prepared the slices using P2, P7 and P14 mice, however the slices made from P14 mice were failed to culture, but slices of P2 and P7 mice could be cultured. In this condition, induction of M1-markers (CD16, CD32, TNF-α and IL-β) by LPS in the slices of P7 mice are significantly higher than slices of P2 mice. The results indicate that the slices made from P7 mice are suitable for evaluate M1-activation. We next investigated the effects of various neurotoxic metals (arsenic(III), triethyltin, lead(II), aluminium(III), manganese(II) and methylmercury) on M1-activation using the improved-organotypic slice culture. Lead(II) and methylmercury increased the expressions of M1-markers, but other metals did not affect. These results suggest that M1-activation may be involved in neurotoxicity induced by Lead(II) and methylmercury. Taken together, the organotypic slice culture of mouse cerebral cortex improved by us can be a useful tool for screening of chemical substances that show neurotoxicity via M1-activation.
Correlations in Metal Concentrations between a Spot and 24-Hour Urine Collection

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The US Department of Veterans Affairs’ (VA) Toxic Embedded Fragment Surveillance Center (TEFSC) uses urine biomonitoring to assess fragment-related metal exposure in war-injured Veterans. While a 24-hour urine collection has long been considered the gold standard for assessing systemic metal exposure, difficulties in obtaining a 24-hour sample, with the potential for external contamination from handling multiple voids during collection in an uncontrolled environment prompted the TEFSC to consider shifting from a 24-hour to a spot urine collection and to conduct an assessment of correlations between the two specimen types. 24 Veterans collected each void over a 24-hour period in separate containers. Concentrations of Al, As, Cd, Co, Cr, Cu, Fe, Pb, Mn, Mo, Ni, W and Zn were measured in each void and a pooled 24-hour sample using inductively coupled plasma-mass spectrometry. Spearman correlations for creatinine-adjusted metal concentrations were determined between each void and the 24-hour sample. Summary correlations were also calculated for each metal across each participant’s observations. On average Veterans submitted 6 samples over a 24-hour period (range: 2-8). Most metal concentrations were within ‘normal’ range. Correlation coefficients between each spot and 24-hour metal concentrations varied greatly according to sample collection time and by metal, with the strongest correlation noted for As (r=0.94, p<0.0001) and the weakest for Cu (r=-0.01, p=0.90). Summary correlations were >0.6 for 7 of the 13 metals. Overall, there were reasonable correlations between spot and 24-hour samples for half of the metals measured, but lack of stronger associations may be partly explained by the relatively low (normal) metal concentrations observed and the expected variability in exposure that can occur over the course of the day from diet and other sources. Evidence suggests that stronger correlations may be observed when urine metal concentrations are elevated above background. Beyond correlation of metal results, other factors suggesting the utility of a spot vs 24-hour sample in surveillance studies include the burden to the Veteran participant, risk for increased contamination, and the range of urine metal concentrations observed.

Neurodevelopmental Aspects of In Utero Exposure to Heavy Metals

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The effects of exposure to heavy metals on child neurodevelopment was evaluated in a Mediterranean cohort of mother-infant pairs. Heavy metals prenatal exposure included mercury, cadmium, lead, and arsenic, as well as essential elements (selenium, zinc, copper) at birth. Cognitive function, language, and motor development were assessed in children at the age of 18 months using the Bayley Scale. Individual metabolic profiles were characterized for untargeted urinary and plasma metabolomics analysis. Integrated pathway analysis and exposome-wide association algorithms were used to identify putative associations between in utero exposure to metals and metabolic pathway dysregulation, as well as between metabolic pathway perturbations and clinical neurodevelopmental indices. Metabolomics analysis revealed the presence of oxoglutaric acid, oxaloacetic acid, succinate, 2-oxoglutarate, formate, isocitrate, oxoglutaric acid, glycerol, L-carnitine, glutathione, methionine, cysteine, pyruvate, N-acetylglutamic acid, β-alanine, serine, and arginine. Pathway analysis revealed that the most perturbed metabolic pathways from exposure to heavy metals were related to the tricarboxylic acid (TCA) cycle; the metabolism of purine, pyrimidine, phospholipids and carnitine; and glycolysis. In addition, in children diagnosed with autism spectrum disorder (ASD), L-arginine of the child, L-tryptophan and acetate of the mother were above the 95th percentile, (5)-Lactate of the child was below the 5th percentile, while child blood levels of As and Hg were above the 95th percentile. Overall, the study provides additional mechanistic evidence on the effect of heavy metals in language, motor and cognitive development (including ASD), through major disturbances to cell biochemistry, which resulted in (a) energy deficiency to developing neurons and (b) the impairment of antioxidant defense mechanisms.

Heavy Metals Contamination in Eye Cosmetics in Alkhjar, Saudi Arabia: A Comparative Study

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Cosmetic products, including eye liner and eye shadow, are widely used for beautification, body care and health purposes. Many countries regulate heavy metals, including cadmium (Cd), lead (Pb), nickel (Ni), cobalt (Co), or chromium (Cr), have been found in all kinds of cosmetics. These metals are known for their toxicity, such as neurotoxic, carcinogenic, mutagenic or teratogenic effects. Continuous assessment and monitoring of heavy metal contamination in cosmetics are needed. This study assessed the heavy metal concentrations in different low-cost brands of eye cosmetics (eye liner and eye shadow) that are sold in local markets in Alkhjar, Saudi Arabia, and compared with them with the heavy metals content in pricey and well-known brands of eye cosmetics. The samples were purchased and prepared in triplicate for analysis then assessed by using inductively coupled plasma mass spectrometry ICP-MS. The experimental results showed that lead (Pb) levels in six different low cost eye-liner and three low cost eye shadow brands ranged from 43 - 547153 ppm and 21 - 104 ppm, respectively, exceeded the maximum acceptable level for Pb as an impurity in cosmetics products based on US FDA and Canadian guidance which is set at 10 ppm, while Pb levels in well-known eye liner brands ranged from only 1 to 2 ppm (within the acceptable range limit). In addition, some analyzed low-cost eye shadow and eye liner cosmetics have high concentration of aluminum, cadmium and barium. In conclusion, there is a potential risk of heavy metal poisoning, particularly Pb poisoning in low-cost eye cosmetic products that are currently available in Alkhjar’s markets. Therefore, it is recommended that further assessment of cosmetics products such as eye liner and eye shadow should be carried out regularly to ensure these particular cosmetics follow international regulations of levels of heavy metals used in their ingredients.

Cytokine Expression Profiles and Metals Exposure Cluster Analysis within a Population Study

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The Navajo Birth Cohort Study (NBCS) was established to address community health concerns about chronic exposure to metals from abandoned uranium mines and waste sites. Many tribal populations experience health disparities, including increased rates of infection, kidney disease, 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 used population samples to look for associations between metal exposure profiles and serum cytokine expression. Samples of whole blood and urine were collected from NBCS participants and analyzed for a panel of 35 metals and for expression of 17 cytokines using multiplex technology. Multiple linear regression revealed significant (p<0.05) associations between concentrations of total arsenic, blood cadmium, and serum copper measured in participants with single cytokines (IFNgamma, IL4, IL5, IL6, and IL13). To begin to better understand exposure patterns and the effects of mixed metals, we used PReMiuM, an R package for Bayesian clustering using Dirichlet processes, to group participants by exposure profiles based on detected metal concentrations. Participants cluster into four distinct groups: low levels of nearly all metals; middling metal values; and two groups in which multiple metals are elevated. In these “high” exposure groups, participants’ metal concentrations measure in the third and fourth quartiles, but the elevated metals differ between the groups. We observe distinct differences in cytokine profiles across the groups, suggesting that metals exposure profiles can influence cytokine expression profiles on a population level. Particularly striking are changes in IL17, IFNalpha, IL29 and IL1beta between the exposure groups. To better understand the potential health effects related to this exposure, it will be important to understand the relationships between chronic mixed metal exposures and potential immune dysregulation.
Absence of Embryofetal Malformations in Rats and Rabbits and Pre/Postnatal Developmental Toxicity in Rats following Oral Dosing with Bictegravir

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Bictegravir (BIC), a potent integrase strand transfer inhibitor (INSTI) with a high barrier to resistance, was first approved in a single tablet regimen BIKTARVY for HIV-1 infection in adults. The potential for BIC to produce developmental toxicity was evaluated in embryofetal development (EFD) studies. A pre/postnatal time point study was conducted to determine the potential effects on maternal pregnancy, parturition, and lactation, and on growth, viability, postnatal development, and reproductive performance of F₀ offspring. In EFD studies, BIC was given orally to pregnant rats (22/group) up to 300 mg/kg/day from gestation day (GD) 7 to 19, and pregnant rabbits (22/group) up to 1000 mg/kg/day from GD 7 to 19. In the PPND study, BIC was given orally to female rats (25/group) up to 250 mg/kg/day from GD 6 to lactation day 20. Clinical observations, body weights, food consumption, and Cesarean, fetal, reproductive and/or postnatal developmental parameters were evaluated.

In the rat EFD study, there was no maternal toxicity and no fetal malformations, including any neural tube defects (NTDs) or developmental toxicity up to the high dose (36-fold AUC exposure margin of 50 mg BIC clinical dose). In rabbits, maternal toxicity occurred at the high dose (1.4-fold AUC exposure margin). No fetal malformations, including NTDs, or increased embryolethality occurred in rabbits; developmental toxicity was limited to fetal weight reductions at the high dose, likely related to maternal toxicity. No overt toxicity as was seen in rabbits has been observed in humans at the clinical dose. In the rat PPND study, the NOEL was the high dose for F₀ maternal toxicity, F₁ pre/postnatal developmental toxicity (including behavior and reproduction), and F₂ early postnatal toxicity, and maternal and pup BIC exposures were 30- and 11-fold, respectively, higher than the clinical dose exposure. Taken together, these data show no direct BIC-related nonclinical developmental or reproductive toxicities.

Diphenyl Phosphate-Induced Toxicity during Embryonic Development


Diphenyl phosphate (DPPH) is an aryl phosphate ester (APE) that is used as an industrial catalyst and chemical additive, and is the primary metabolite of flame retardant APEs, including triphenyl phosphate (TPH). DPPH has been detected within urine of pregnant women, underscoring the need for developmental toxicity data. The objective of this study was to determine the potential in-UTR-induced toxicity during embryonic development. Using zebrafish as a model, we relied on phenotyping, hemoglobin quantification in situ, and mRNA-sequencing to determine the potential impacts of DPHP on cardiac morphogenesis, red blood cell formation, and the transcriptome, respectively, following whole-embryo exposures from 24-72 h post-fertilization (hpf). As DPHP and TPHP co-occur within environmental media, we also conducted a bivariate mixture experiment to determine if exposure to DPHP (250, 500, or 1000 µM) in the presence or absence of TPHP (5, 10, or 20 µM) - a cardiotoxic APE - in developing zebrafish - resulted in additive or synergistic toxicity. While exposure to DPHP from either 24-72 or 30-72 hpf resulted in a significant decrease in red blood cells (RBC) in the distance between the sinus venosus and bulbus arteriosus (SVBA), the magnitude of this effect was significantly decreased following initiation of exposure at 48 hpf - a developmental stage that follows cardiac looping. Based on mRNA-sequencing, exposure to 1000 µM DPHP from either 24-30 or 48-48 hpf resulted in significant alterations in mitochondrial function and heme-binding pathways relative to controls. Indeed, hemoglobin quantification in situ revealed that DPHP exposure from 24-72 hpf resulted in a significant decrease in hemoglobin relative to controls, suggesting that DPHP may suppress proliferation and/or maturation of erythroid precursors into erythrocytes. Co-exposure of embryos to DPHP and TPHP from 24-92 hpf resulted in a significant increase in SVBA length relative to DPHP or TPHP alone, suggesting that exposure to DPHP-TPHP mixtures may lead to additive effects on cardiac development. Overall, our findings to date demonstrate that DPHP is biologically active during embryogenesis, albeit the potency of DPHP is ~100-fold less than TPHP based on whole-embryo expression exposures - a finding that is consistent with the US EPA’s ToxCast data and likely due to DPHP’s high water solubility (log Kow = 1.4) and, as such, limited uptake into developing embryos.

Snus Smokeless Tobacco Impacts Osteotoxicity by Repressing Cellular Stress Mechanisms through the AKT-Signaling Network

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In the last 20 years, tobacco-related health disparity concerns have shifted focus from smoking to other forms of tobacco use. Pregnant women struggling with nicotine addiction may switch to harm-reduction tobacco products (HRTPs) as HRTPs are marketed as less harmful to users. Contrarily, epidemiological studies suggest that HRTPs may hinder infant bone mineral density. While current research indicates HRTP exposure directs targeted osteogenesis as gestational exposure of mouse embryos reduced skull mineralization by a presently unknown mechanism. To model osteoblocytopathy, human embryonic stem cells were differentiated into osteoblasts and consequently exposed to conventional sidestream cigarette (CSC) or a commercial Snus smokeless tobacco extract. Snus exposure decreased osteocalcin differentiation at subtoxic levels, while CSC exposure inhibited osteogenesis at cytotoxic levels. Both showed significantly increased cellular reactive oxygen species at inhibitory doses. Notably, dosing cultures with H2O2 alone inhibited osteogenesis only when dosing occurred during early differentiation. Cellular stress assessment found CSC cultures featured more apoptotic cells while Snus cultures had more injured cells without active apoptosis. Mechanistic analysis revealed higher activation of the survival kinase AKT exclusively in Snus-treated cultures. Activity of the stress rescue kinase, JNK, was also reduced in Snus cultures. Rescue experiments found concurrent AKT inhibitor or JNK activator treatment to rescue osteocenesis in Snus-treated cultures implicating mis-regulation of these kinases in Snus inhibitory outcomes. Global proteomic analysis of AKT signaling pathway targets identified hyperphosphorylation of FOXO transcription factors—responsible for bone homeostasis in adult cells—exclusively in Snus cultures marking them for nuclear exclusion. Taken together, our data suggests that Snus products impart normal osteogenesis through distinct mechanisms with Snus potentially operating through a mechanism specific to early osteogenic commitment.

Adverse Developmental Effects in Progeny of Zebrafish That Were Exposed to Atrazine during Embryogenesis

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Atrazine (ATZ) is an herbicide that is commonly used on crops in the Midwestern US. ATZ has a long half-life and is a common contaminant of drinking water sources. Although the US EPA set the maximum contaminant level at 3 parts per billion (ppb), water sources often exceed this limit. ATZ has been implicated as an endocrine disrupting chemical in multiple species. The current study used the zebrafish model system and tested the hypothesis that an embryonic parental ATZ exposure altered protein expression, morphology and behavior in developing progeny. Zebrafish embryos were collected from adults that were exposed to 0, 0.3, or 30 ppb ATZ during embryogenesis. Protein, morphology, and behavior analysis was completed with offspring from each chemical treatment group aged 120 hours. The offspring received no additional chemical treatment. Proteomic analysis found that parental exposure to ATZ, regardless of dose led to differential expression of proteins involved in pathways of cancer; neurological disease; and morphology of tissue, and muscular development. Morphology measurements of total length, head width, and brain length were not significantly different (p>0.05). Head length was found to be significantly different between the control group and parental exposure groups, with increasing exposure leading to increasing head length (p<0.05). The light/dark behavioral test detected no significant differences in swimming distance, velocity, or time spent moving/not moving (p>0.05). These findings suggest that a single embryonic parental exposure can alter proteomic expression and morphology in progeny supporting further assessment of adverse impacts in this generation.

Role of the Transcription Factor Nfe2 and Pro-oxidant Exposure in Inner Ear Development in Zebrafish

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Millions of people worldwide suffer from hearing loss. While several mechanisms have been associated this loss, the role of oxidative stress remains underexplored. Nfe2, a transcription factor in zebrafish and other vertebrates, is localized to the otic vesicle during development and has been shown to me-
2391 Chemical-Induced Craniofacial Abnormalities Share a Conserved Molecular Mechanism with Neural Crest Development in Zebrafish and Mammals


Alternative methods for testing the developmental toxicity of chemicals have been developed worldwide. Zebrafish embryos represent a promising model for assessing teratogenicity owing to their rapid development and transpar-

ency. Many craniofacial abnormalities is a critical endpoint for eval-

uating chemical-induced teratogenicity, the common ontogenic pro-

cess disturbed by teratogens in zebrafish and mammals has not been wel-

l studied. In the current study, we exposed zebrafish embryos to 13 recog-

nized teratogens (retinoic acid, methotrexate, salicylic acid, valproic acid, caffeine, warfarin, methanol, aspirin, isoniazid, dexamethasone, 5-fluouracil, phenytoin, thalidomide, boric acid, and imatinib) and then performed cartilage staining using Alcian blue to examine detailed craniofacial morphological changes induced by the teratogens. Most of the teratogens induced craniofacial mal-

formations, which were found in both the neurocranial cartilage and pharyn-

gal skeleton. Retinoic acid, methotrexate, salicylic acid, and valproic acid severely abrogated the development of the ethmoid plate, mandible, and skull base. Caffeine, warfarin, hydroxyurea, and dexamethasone resulted in moderate phenotypes, namely obvious separated ethmoid plate and short-

ening of Meckel’s cartilage. The other compounds induced slight craniofacial mal-

formations of the lower jaw and skull base. Notably, these observations partly corresponded to the abnormalities reported in mammals, suggesting that the mechanism by which teratogens disrupt cranial neural crest (CNC) development in zebrafish and mammals is common. Comprehensive and semi-quantitative analyses were performed to examine the conserved biol-

genetic pathway involved in CNC development between zebrafish and mammals. We elucidated that the disruption of CNC development is the target biological pathway underlying chemical-induced craniofacial mal-

formations, and this finding represents a promising molecular endpoint for mechanism-based teratogenicity evaluation.

2392 Filling Gaps by New Approach Methods: A Case Study for Teratogenicity Prediction of VPA Analogs

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In the last years new approach methods (NAM) have been developed to allow animal-free hazard assessment. They are often based on human cell cultures and they measure fundamental biological processes, such as neurite outgrowth, neural differentiation or cell migration. NAM are useful to assess whether chemicals affect key event (KE) as part of an adverse outcome path-

way (AOP). The regulatory use of data generated from NAMs represents the next big challenge in the field of in vitro toxicology and a first step may be the use of NAM for readacross (RAX) purposes. Here, we present a RAX case study using 8 structural analogs of valproic acid (VPA), an anticonvulsant drug with known in vivo teratogenic activity. Literature research revealed that 3 compounds are in vivo positive, 3 show no teratogenic in vivo effects. 2 com-

pounds have an unknown in vivo activity. This shows that a simple structural similarity may not be sufficient to conclude on teratogenicity. To improve RAX, we considered activity-activity relationships within an overall QSAR ap-

proach, added data on the mode of action (MoA), and complemented this information with data from NAM that measure disturbed embryonic devel-

opment. For the latter we used the zebrafish embryo test (ZET), the mouse embryonic stem cell test (mEST) and a recently developed induced pluripo-
tent stem cells (iPSC)-based neurodevelopmental model (UKN1). One MoA of VPA is the inhibition of histone deacetylases (HDAC). We found that the compounds showing a strong HDAC inhibition correlate with positive hits in our NAM. The UKN1 assay is an in vitro test method that models early neu-

rodevelopmental changes on human iPSC differentiating into neuroectodermal progenitor cells. These cell culture allows the measurement of 3 important endpoints; cell viability; gene expression and the formation of neural rosettes as a functional differentiation endpoint. With this combination of our end-

points we have identified all in vivo positive compounds and 2 out of 3 negative compounds. With the approach we applied the application of NAM for RAX studies, and point to a strategy that uses (i) existing in vivo and in vitro data; (ii) incorporates QSAR and (iii) and also provides new data to fill prediction gaps. The combination of these three aspects delivered a good prediction of teratogenicity of the investigated VPA analogs.

2393 Placental Transfer of 125I-Labeled Humanized Immunoglobulin G2ΔA in the Cynomolgus Monkey


Antibody-like biopharmaceuticals cross the placenta by utilizing existing transport pathways (e.g., FcRn receptor) for transfer of maternal antibodies to the conceptus. There are limited data evaluating this transfer during organogenesis in any species. Understanding placental transfer of antibody-like biopharmaceuticals can help predict risk of developmental toxicity across species, including humans. To complement previously published placental transfer data in the rat with humanized IgG2ΔA (hIgG2ΔA), the timing and magni-

tude of transfer in the cynomolgus monkey and embryo/fetal biodistribution of maternally administered 125I-radioiodinated hIgG2 was quantified on gesta-

tion days (GD) 35, 40, 50, 70 and 140 using gamma counting and whole body autoradiography 24 hours following intravenous injection. Chorallantoic or placental tissues were collected at all time points for Western Blot analysis with anti-FcRn antibody. Maternally administered 125I-hIgG2 was found in monkey embryo/fetal tissues at all time points, including during organo-

genesis (until GD 50). Due to embryo size, embryonic blood samples were not available at GD 35, but 125I-hIgG2 was detectable from GD 40 onwards. Embryo/fetal plasma 125I-hIgG2 concentration increased during gestation, but only increased slightly up to GD 70 in embryo/fetal tissues, with hIgG2 tissue concentrations generally similar between GD 70 and 140. The embryo/fetal/maternal 125I-hIgG2 plasma concentration ratio was approximately 2.3 fold higher on GD 140, in comparison to ratios on GD 40. Importantly, pla-

cental FcRn protein expression was confirmed at all timepoints. These data demonstrate placental transfer of hIgG2 in a nonhuman primate model, and at levels comparable to the rat model. Hence, rats also represent a biologically and clinically relevant species for placental transfer of hIgG2. Antibody was measured in the nonhuman primate and rat embryo/fetus during specific de-

velopmental windows, including organogenesis. Thus, direct antibody bind-

ing to biological targets present in the developing conceptus is conceivable, raising the potential for adverse developmental outcomes.

2394 Identifying the Molecular Mechanisms Responsible for Persistent Effects of Developmental Exposure to Chlorpyrifos on Behavior

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Chlorpyrifos (CPF) is one of the most widely used organophosphorus insec-

cides (OPs). Developmental exposure to OPs has long-lasting negative im-

pacts, including abnormal emotional behavior. These negative impacts are observed at levels that cause only minimum inhibition of acetylcholinester-

ase, the canonical target of OPs. However, exposure to these levels results in the inhibition of the endocannabinoid metabolizing enzyme fatty acid amide hydrolase (FAAH) and altered emotional behavior specifically increased so-

cial play. However, the molecular mechanisms responsible for this increased social play are not known. In this study, male rat pups were exposed orally to either corn oil, 0.75 mg/kg CPF, or 0.02 mg/kg PF-04457845 (PF; a specific

hydrolase (FAAH) and altered emotional behavior specifically increased so-

cial play are not known. In this study, male rat pups were exposed orally to either corn oil, 0.75 mg/kg CPF, or 0.02 mg/kg PF-04457845 (PF; a specific
testing, the amygdala was collected from each cohort and protein expression was determined using a label-free shotgun proteomic approach. The obtained differentially expressed proteins from the different cohorts were analyzed by DAVID and Ingenuity Pathway Analysis software. The data from the non-behavior group of rats suggest that FAAH inhibition by development- tial exposure to CPF and PF persistently affects glutamatergic and GABAergic signaling. These data also suggest that there is a similar pattern of protein expression between CPF and PF suggesting that these long-term effects are due to the inhibition of FAAH. The data from the behavioral group of rats suggest that alterations in the glutamatergic and GABAergic signaling and activation of opioid signaling could be responsible for the increased social play behav- ior. These alterations in neurotransmitter signaling were observed in both CPF and PF treated rats. Overall, the data suggest that FAAH inhibition by CPF leads to an alteration in the opioid, glutamatergic, and GABAergic signaling that could be responsible for increased levels of social play.

Assessing the Toxicological Response of Placenta to PFAS Using “Placentox”—A Novel High-Throughput mRNA Biomarker Platform

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Per- and poly-fluoroalkyl substances (PFAS), also known as fluorosurfactants, are a group of chemicals that have been widely used in stain repellents, paints, polishes, protective coatings, and firefighting foams. While some of these chemicals, including perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), have been discontinued in the United States, their persistence in the environment and continued use internationally make them a toxicological concern. Moreover, GenX, a modern fluorosurfactant replace- ment for PFOA, has recently contaminated drinking water in the Cape Fear River in the Wilmington area, warranting immediate evaluation of the risks associated. The USEPA acknowledges that certain PFAS can cause reproductive, developmental, liver, kidney, and immunological toxicity and may even be carcinogenic. While prior reports have shown that PFAS can cross the pla- centa, there is little understanding of how this class of compounds can impact placental function itself. We therefore developed the “PLACENTOX”-a mRNA biomarker panel designed to comprehensively examine potential changes in placental homeostasis, development, and function as a result of PFAS exposure. This panel includes 90 well-established mRNA markers of trophoblast fusion, invasion, nutrient and drug transport, apoptosis, proliferation, inflam- mation, oxidative stress, hormones production, and unfolded protein response in tandem with the Fluidigm Biomark HD platform for high-throughput qPCR analysis. JEG-3 cells, an in vitro trophoblast cell line, and ex vivo explants isolated from human term placentas were treated with PFOA, PFOS, and GenX (10-1000 ng/mL) for 24 hours and mRNA expression was quantified using this technique. Selected genes showing significant (p < 0.01) changes for one or more PFAS were confirmed using traditional qPCR in a 96 well plate format, and protein expression was analyzed by western blot. Ultimately, this panel can be utilized in future studies to provide a comprehensive analysis of placental toxicity for any environmental toxicant of concern.

Placental Redox Potential and Histiotrophic Nutrition in Organogenesis—Stage Mouse Conceptuses Treated with Valproic Acid

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Neural Tube Defects (NTDs) comprise a group of morphological birth defects with unknown etiology. Exposure to anesthetics drugs including valproic acid (VPA) increases NTD incidence by mechanisms that implicate increased reactive oxygen species but not induction of the protective Nrf2 antioxidant response. This mouse whole embryo culture study (gestational days [GD] 8-9) explores redox mechanisms of VPA on neural tube closure. We hypoth- esized that pre-treatment of conceptuses with 1,2-dithiole-3-thione (D3T), a synthetic Nrf2 inducer, on GD8 would increase glutathione (GSH) and elicit a more negative, protective, GSH/GSSG redox potential (Eh) across the develop- mental time course. HPLC measurements of GSH and glutathione disul- fide (GSSG) indicated an increased Eh from -217.71mV to -173.6mV following a 14hr VPA exposure in embryos that was significantly different from both the control and the co-treated D3T+VPA group. At 20hr, Eh of control versus D3T+VPA increased in the embryo (-225.28mV to -152.49mV) and in visceral yolk sac (-204.89mV to -135.74mV). Total GSH (GSH+2GSSG) concentrations decreased significantly at 20hr from 4.8mM in the control embryos to 1.8mM in the D3T + VPA embryos. Altered GSH status and increased Eh produced indirectly through decreases in protein uptake and degradation (histiostrophic nutrition pathways, HNP) were also evaluated. Clearance of FITC-Ab from the culture medium at 1, 3, or 6hrs on GD8 was measured for all exposure con- ditions. HNP activities in the VPA and D3T+VPA groups were decreased between 32-55% at 1 and 3hr but were variable and not statistically significant. The HNP results do suggest that VPA may have acute early effects on HNP activity that is also not correlated with cellular redox activity. These results indicate that VPA exposure does not produce extensive, uniform, depletion of total GSH or increased Eh, in all conceptal tissues during organogenesis and that Nrf2 induction by D3T is sufficient alone or with VPA to protect against VPA-induced changes. Previous evidence implicating increased reactive oxygen species with higher incidence of neural tube defects following VPA exposure was not shown to be correlated with the conceptual GSH redox state but will require a more directed spatial and temporal analysis of oxidative post-trans- nential protein modifications.

Maternal Bococizumab (Anti-PCSK9 Monoclonal Antibody) Administration Does Not Affect Rat Pre- and Postnatal Development

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Bococizumab is a humanized monoclonal IgG2a antibody against propro- tein convertase subtilisin/kexin type 9 (PCSK9) for the treatment of hyper- lipidemia. In a previous study conducted in rats, there were no effects on embryo-fetal development in the presence of decreased maternal and fetal cholesterol. In a pre- and postnatal development study bococizumab was ad- ministered intravenously to pregnant Sprague-Dawley (SD) rats (n=22/group) at 0, 10, 30, and 100 mg/kg once every three days beginning on Gestation Day 6 and continuing through Lactation Day 20. The doses evaluated were previously demonstrated to decrease maternal and fetal cholesterol during gestation in a dose-dependent manner. Bococizumab was well tolerated and no area, warranting in F0 females or any natural delivery parameters. In addition, there were no clinical signs or effects on body weight, food consump- tion, sexual maturation, behavioral endpoints (motor activity, Morris water maze, and acoustic startle testing), or reproductive endpoints in the F1 rats. This study has demonstrated no adverse effects on pre- and postnatal development at doses of bococizumab that cause reductions in maternal and fetal cholesterol.

Role of Nrf2a in Modulating MEHP-Induced Hepatosteatosis following Embryonic Exposure in Danio rerio

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Di(2-ethylhexyl) phthalate is a plasticizer and ubiquitous human toxicant. Its bioactive metabolite, mono(2-ethylhexyl) phthalate (MEHP) has been linked to abnormal development, increased oxidative stress, and metabolic syn- drome—namely obesity. Nrf2 is a transcription factor that regulates gene expression through Antioxidant Response Elements (AREs) in the promotor- ers of genes and functions as an inducible regulator of oxidative stress. The objective of this study was to investigate the role of Nrf2a in juvenile adi- posity following embryonic exposure to MEHP. Zebrafish (Danio rerio) wild type (wt) and mutant (m) embryos from a Nrf2a mutant line were exposed to 0 or 200 μg/L MEHP through immersion beginning at 6 hours post fertilization (hpf) and concluding at 120 hpf. At 120 hpf fish were placed in clean system water and maintained to 15 days post fertilization (dpf). At 15 dpf fish were imaged for length and stained with Oil Red O to visualize lipid depots. Wild type control fish were significantly longer than mutant control fish. Developmental MEHP exposure significantly increased hepatosteatosis in both wild type and Nrf2a mutant fish, an effect that was exacerbated in mutant fish. Adipogenesis was moderately increased in fish exposed to MEHP, though this trend was not statistically significant. These data indicate develop- mental exposure to MEHP may increase risk for hepatosteatosis, and that impaired Nrf2a function may exacerbate this phenotype. This result is consis- tent with Nrf2a’s previously reported facilitation of hepatoprotection in adults. Our data suggest that toxicant-induced oxidative stress during embryonic development is a risk factor for hepatosteatosis later in life, and Nrf2 function is important for mitigating this stress and decreasing risk for hepatosteatosis.
Sensitivity of Micro-Computed Tomography (micro-CT) Imaging to Visualize Skeletal Variations and Malformations in Embryo-Fetal Development Toxicity Studies


Embryo-fetal developmental (EFD) toxicity studies are an essential means for determining the potential effects of a drug on the developing fetus. Traditionally, these studies utilize the Alizarin red staining method to identify variations and malformations in the fetal skeleton. Wise et al. (2013) determined that micro-CT using spatial resolution (~60 µm) was able to visualize small skeletal structures in a fetus when compared to Alizarin red staining. However, small elements of ossification identified in staining were unseen by micro-CT. In an unpublished, in-house study, it was demonstrated that micro-CT, using high resolution (35 µm pixel), could visualize small skeletal structures in a fetus, including small degrees of ossification. The purpose of this study was to utilize a developmental toxicant known to induce skeletal malformations and variations in early organogenesis and without overt systematic toxicity, and to compare the skeletal findings between the Alizarin red staining and micro-CT imaging methods. Therefore, Acetylalsaliclyc Acid (aspirin) was chosen as the test item. Forty-four time-mated, Sprague Dawley rats (22/group) were dosed with either vehicle control (0.2 % carboxymethyl-cellulose in water) or 500 mg/kg of aspirin on Gestation Day (GD) 9. Cerean sections were performed on GD 21 and the fetuses were euthanized. Micro-CT imaging was performed on the rat fetuses at 35 µm pixel size. Following micro-CT imaging, the same fetuses were eviscerated and manually processed using the Alizarin red staining method. Skeletal anomalies of the ribs, sternebrae, and thoracic vertebrae, including ossification defects that were observed from the Alizarin red stained fetuses were also observed in micro-CT imaging, utilizing both 2D and 3D images. In conclusion, micro-CT imaging was demonstrated to be sensitive enough to visualize rat fetus skeletal assessments. Thus, supporting the use of micro-CT imaging for replacement of Alizarin red staining in developmental studies. Replacing traditional staining with micro-CT imaging would allow for high throughput skeletal imaging with reduced processing time, reduced hazardous waste production, and reduced archival products associated with traditional staining.

Validation of a Novel Human Stem Cell-Based Gene Expression Assay for In Vitro DART Assessment


Testing for developmental and reproductive toxicology (DART) is a crucial part of the toxicological risk assessment. Today, DART mostly relies on animal testing. However, an alternative in vitro test, such as embryonic stem cell differentiation-based assays, are increasingly being used. However, these in vitro assays often do not provide mechanistic insight and the results are difficult to translate to human risk due to inter-species differences. To improve in vitro identification of developmental toxicants, we identified 43 potential biomarkers in human induced pluripotent stem cells (hiPSC), marking different developmental stages from stem cells to mature tissues. To test whether compounds affect development, first we optimised the differentiation protocols for hiPSC towards cardiomyocytes, hepatocytes and neural rosettes and confirmed the expression of selected biomarkers (OCt4, BMP4, MYH6, FOXA2, SOX17, AFP, ALB, SOX1, PAX6) by qPCR. During differentiation, expression of the developmental stage marker OCT4 decreased, while expression increased for matured tissue markers MYH6 in cardiomyocytes, ALB and AFP in hepatocytes and PAX6 and SOX1 during neuronal rosette differentiation. Next, we exposed differentiating cells to 15 different compounds (both known teratogenic compounds as well as non-teratogenic compounds). We observed a downregulation of the cardiomyocyte-specific biomarker MYH6, hepatocyte-specific markers ALB and AFP and neural specific biomarker PAX6 during teratogenic compound treatment. The cardiomyocyte-specific biomarker PAX6, the hepatocyte-specific markers ALB and AFP, and the neural specific biomarker PAX6 were significantly downregulated at 100 µM, while the other treatments did not show differences. Blastocyst formation was reduced to only 24% at 1000 µM MEHP, while hatching initiation was decreased by 1% and 100 µM MEHP. Fully hatched embryos were sharply reduced to just 1.33% with 1000 µM MEHP, while the other treatments did not show differences. The diameter and volume of the hatched ones were similar between groups. The ratio of inner cell mass to trophectoderm cells appeared to be more pronouncedly affected at the low concentration of 1 µM MEHP, and was increased, although statistical analysis was not yet done. In conclusion, MEHP has negative effects on preimplantation embryonic development at various stages, depending on the dose, and further studies need to be carried out.

Bisphenol A Exposure Differentially Affects Echinoderm Embryogenesis

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For many the ocean is a wonderful peaceful oasis but for marine invertebrate larvae it can be a harsh and stressful journey to adulthood. Larvae are exposed to numerous environmental stresses resulting from both nature and anthropogenic effects; such stresses include temperature change, oxygen availability, and environmental toxicants. One such environmental toxicant is Bisphenol A (BPA), a plasticizer found in such products as plastic bottles, cans, electronics and medical devices, which acts as an endocrine disruptor thus causing fertility and developmental defects. Increased ocean pollution with items containing BPA poses a serious hazard to marine life. Therefore, we investigated the effect of BPA on embryogenesis of the sea star, Patiria miniata. Eggs and sperm were treated with BPA (0, 1, 5, 10 µM) 30 minutes before fertilization and analyses were performed on developing embryos 24 and 48h post-fertilization. We found that BPA exposure significantly increases nascent protein synthesis and mitochondrial activity of P. miniata embryos. Additionally, we performed the same BPA treatment on the sea urchins, Lytechinus variegatus and Strongylocentrotus purpuratus. BPA exposure significantly decreased mitochondrial activity and nascent protein synthesis of L. variegatus embryos but did not affect nascent protein synthesis of S. purpuratus embryos. Our findings suggest that BPA exposure differentially affects sea stars and sea urchins and that not all species of echinoderms respond the same to the environmental stress of BPA exposure.

Mono(2-ethylhexyl) Phthalate (MEHP) Adversely Affects Mice Embryonic Development In Vitro

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MEHP is the first active metabolite from the breakdown of DEHP, which is used to provide flexibility to plastics and is commonly found in food packaging, personal care products and medical supplies. MEHP is an endocrine disrupting chemical, and its high concentration in the body has been linked with negative reproductive outcomes. The objective of this research is to investigate negative effects of MEHP on early embryonic development using an in vitro model. Embryos were collected from the oviduct of superovulated CD-1 female mice mated with C57BL/6 males. Six to ten embryos per group were cultured in 30 µl drops of either KSOm cell medium, vehicle (DMSO 0.005% or 0.05%) or 0, 1, 10, 100 and 1000 µM MEHP and were observed daily over six days. After, diameters of embryos were measured, and blastocysts were fixed for differential staining of inner cell mass (marked by Oct-4) and trophectoderm (marked by CDX-2) by immunofluorescence. Analyses of blastocysts and cell types were done with a confocal LSM 880 microscope and Imaris Software. Percentage data were analyzed by chi-square and Fisher’s exact test considering Bonferroni adjustment. Continuous variables were tested for normality and compared against control by one-way ANOVA and Tukey’s post-test or Kruskal-Wallis test and Dunn’s post-test. Statistical analyses were done in GraphPad Prism 7.0 and p < 0.05 was considered significant. The percent of embryos that developed to the 2-cell stage was significantly reduced by 1% and 100 µM MEHP. Percentage reaching 4-cell stage was reduced by 1, 10 and 100 µM MEHP. The 10 µM MEHP also had a detrimental effect at 8-cell and morula stages. Blastocyst formation was reduced to only 24% at 1000 µM MEHP, while hatching initiation was decreased by 1% and 100 µM MEHP. Fully hatched embryos were sharply reduced to just 1.33% with 1000 µM MEHP, while the other treatments did not show differences. The diameter and volume of the hatched ones were similar between groups. The ratio of inner cell mass to trophectoderm cells appeared to be more pronouncedly affected at the low concentration of 1 µM MEHP, and was increased, although statistical analysis was not yet done. In conclusion, MEHP has negative effects on preimplantation embryonic development at various stages, depending on the dose, and further studies need to be carried out.

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2403 Modulation of Peroxisome Proliferator-ACTivated Receptors Gamma (PPARγ) Signaling Perturbs Embryonic Pancreas Development in the Zebrafish, Danio rerio

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Peroxisome proliferator-activated receptors (PPARs) are essential transcription factors for glucose and lipid homeostasis, as well as critical development processes such as adipocyte differentiation. Differentiation of preadipocytes into adipocytes is modulated by PPAR gamma (PPARγ) - demonstrating the formation of ectopic adipocytes and abnormal birth weight when altered. Toxicants such as phthalates, butylparaben, and PFOS are reported to have affinity for PPARγ, and have been previously shown to elicit adverse effects on the developing zebrafish (Danio rerio). This begs the question of how disruption of PPAR activity during development may alter fetal metabolic processes that are carried into adulthood, such as in the pancreas. Here we examine the effects of altered PPARγ activity on the developing exocrine and endocrine pancreas in zebrafish embryos. Transgenic fish lines that express GFP in beta cells of the endocrine pancreas (Tg(ins:GFP)) or in ptf1a expressing endocrine cells of the exocrine pancreas (Tg(ptf1a:GFP)) were used to assess the impact of PPARγ modulation on pancreas development in live zebrafish embryos. Beginning at 24 hours post fertilization, embryos were exposed daily to either Rosiglitazone (selective PPARγ agonist) or T0070907 (selective PPARγ antagonist) at concentrations of 0 (0.01% v/v DMSO), 0.1, 1, and 10 µM. At 96 hpf, embryos were imaged live with brightfield and fluorescent microscopy, and images were analyzed using morphometrics. Fish length and yolk sac utilization suggests that morphological delays were associated with 10 µM T0070907 and Rosiglitazone exposures. T0070907 exposure correlates with a decrease in islet area but demonstrates a u-shaped dose curve response in exocrine pancreas length, while Rosiglitazone appears to only alter endocrine pancreas area in a non-dose dependent manner. However, Rosiglitazone results in an increased occurrence of endocrine pancreatic deformities as 21% of 10 µM exposed embryos display a ‘wispy’ or ‘curved’ pancreas, when compared to 0% of controls. In addition, a 7% increase in fragmentation of endocrine pancreas islets was observed in both 1µM and 10µM Rosiglitazone exposed embryos, compared to controls. These findings suggest that pancreas development is sensitive to modulation of PPARγ, but it does not fully recapitulate the morphologies observed with preclinical studies. This work is being supported by grant funding from F32ES028085 and NIH RO1ES025748.

2404 Improving In Silico Predictions in Developmental and Reproductive Toxicology

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In silico predictions for toxicity are able to assist risk assessors with chemical safety assessments. The confidence in a specific method can dictate whether the approach is used to support compound prioritization or for regulatory decision-making. Developmental and reproductive toxicity (DART) continues to be a challenge for in silico predictions, partially due to a lack of data availability across a broad chemical space. While there is a large amount of publicly available data, a significant amount of information from DART studies, including those conducted by pharmaceutical companies, remains in confidential locations. In an attempt to broaden the chemical space for DART in silico evaluations, data from embryo-fetal development studies in rats and rabbits was curated by Bristol-Myers Squibb (BMS) and provided to Lhasa Limited for evaluation. The data consisted of 22 compounds with publicly available structures and known pharmacology and was shared with Lhasa Limited for evaluation using in silico tools. One goal of this initiative was to potentially uncover new structural alerts for incorporation into Derek Nexus’ knowledge base, as well as strengthening existing alerts. Evaluation of the dataset alongside publicly available data, was unable to generate new structural alerts predicting for reprootoxicity. However, molecular initiating events of interest for DART were identified for potential incorporation into an adverse outcome pathway (AOP) framework. For example, the development-toxicity associated with tyrosine kinase inhibitors, can be rationalized in terms of their potential to disrupt the receptors’ normal function during organogenesis. Subsequent literature review of relevant mechanistic studies of the prioritized targets enabled synthesis of new putative AOPs. AOPs such as these are able to document existing evidence to support a pathway leading from a molecular initiating event (MIE) to an adverse outcome such as embryo lethality, teratogenicity, or impaired fertility. Documenting the evidence in this way can ground relevant in silico models and provide scientific rationale of their output to risk assessors, thus supporting better decision-making. This data sharing initiative has highlighted some chemical space of concern for DART, which in silico approaches should cover, in order to increase user confidence in this setting.

2405 Toxicity Interaction of the Two Most Common Agricultural Herbicides in the United States: Glyphosate and Atrazine

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Glyphosate and atrazine are the two most commonly used agricultural herbicides in the United States. These herbicides are sprayed on crops to reduce weed populations, but move into drinking water sources following rain events. There is a lot of controversy surrounding the use of these two herbicides because of suspected hazardous health effects. The US EPA established the maximum contaminant level (MCL) of glyphosate in drinking water at 700 ppb (µg/L) and the IARC designated glyphosate as “probably carcinogenic to humans.” Though atrazine has been suspected of being a carcinogen, the main health concerns surround endocrine disruption with the MCL set at 3 ppb. Though there are countless studies concerning the individual toxicity of these herbicides, there is limited research on the potential toxic interaction making this a substantial gap in our knowledge. This study aims to test the hypothesis that a toxicity interaction occurs between atrazine and glyphosate at treatment concentrations below those observed for the single chemical exposure. This study in conjunction with previous research by our group, first identified developmental toxicity profiles of the individual herbicides using the zebrafish model system. This data was then used to inform on treatment concentrations to evaluate mixture interactions on behavioral and morphological outcomes. Zebrafish were bred and their embryos immediately collected and exposed to one of four treatments, which included the MCL for each herbicide alone or in mixture since single chemical analysis indicated changes in behavior and morphology at concentrations 10x the MCL for each herbicide. Currently, at 120 hours post fertilization the behavior results showed that all treatment groups were significantly different from the control (p<0.05; n=4 with 32 subsamples/biological replicate). The morphological data including tail length, head length, head width, and brain length were not significantly different in the mixture exposure (p>0.05; n=7 with 11-12 subsamples/biological replicate).

2406 Patterns of Hyaluronic Deposition in Normal and Diseased Pediatric Livers

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The incidence of obesity and non-alcoholic fatty liver disease (NAFLD) is growing in children. Hyaluronan (HA), an extracellular matrix (ECM) glycosaminoglycan, is increased in liver and blood from adults with advanced liver disease. HA accumulation is also associated with fibrosis and fibrosis in extrahepatic tissues. HA levels have not been examined in healthy or diseased livers from pediatric patients. Here, we sought to determine if hepatic HA deposition was associated with donor age and/or disease by using a pediatric liver tissue microarray (TMA). The TMA contained 25 tissues from donors aged 4 months to 18 years old and 5 adult controls (3 mm diameter cores, 4 µm-thick array). Donor demographics and health data were collected from interviews with next of kin and hospital records. Three consecutively cut arrays were stained with hematoxylin and eosin (H&E) and Gomori’s trichrome for evaluation of tissue histology, and collagen, respectively, or with HA binding protein (HABP) to localize HA. Images of the arrays were obtained with a Nikon HCA system and a Hamamatsu Orca Flash 4 camera (HABP) or a Nikon DS-F13 camera (H&E and Gomori’s trichrome). HA was detected in 24 pediatric and in 5 adult control tissues. Well-defined HA staining was observed in the ECM-rich portal tracts while thin but distinct rims of HA were observed surrounding central veins. These observations were independent of donor age. HA was also observed in livers from patients with histological evidence of fibrosis. Moreover, in overweight or obese patients with NAFLD and fibrosis, typical HA-positive regions were expanded, more diffuse, and also found surrounding steatotic hepatocytes outside of portal tracts. There was variability in this staining pattern as this assessment was complicated by micro- and macrovesicular steatosis ranging 50 - 80% in the NAFLD patients. In tissues deemed normal or near normal based on microscopic examination and medical history, the HA staining was less intense than in diseased tissues independent of donor age. The pediatric liver TMA allowed demonstration of normal and disease-associated HA localization and the identification of distinct staining patterns in different patient cohorts.
Advanced paternal age is associated with elevated risk for a constellation of somatic and neuropsychiatric diseases in offspring. We recently demonstrated that offspring of aged male mice exhibit accelerated aging across most organ systems, neuroinflammation, and reduced survival. Sperm from aged fathers and brain tissue from old father offspring displayed enhanced mammalian target of rapamycin (mTOR) activation suggesting that offspring of aged fathers age faster due to inherited mTOR hyperactivity, and that they could thus be more susceptible to exogenous stressors implicated in neurodegenerative processes. To test this hypothesis, we prepared primary cortical neuron cultures from embryos collected from young pregnant females that were mated to young (less than 24 weeks old) or old males (over 90 weeks old). In cortical neuron cultures from the two conditions, we compared baseline and rotenone-induced alterations in cytotoxicity, and in live-cell imaging experiments evaluated reactive oxygen species generation, and electrophysiological activity by means of calcium imaging. In line with our predictions, neurons from old father offspring showed enhanced susceptibility to rotenone-induced cytotoxicity, and showed a baseline and rotenone-induced enhancement of mitochondrial superoxide production. Calcium imaging revealed reductions in calcium burst activity in old father offspring neurons, without apparent cytotoxicity or impairments in depolarization in response to potassium chloride (KCl). Our results suggest that paternal age can influence offspring risk for brain aging and neurodegeneration, likely through baseline mTOR hyperactivation, as well as exaggerated responses to environmental stressors.

In Vivo Evaluation of Bisphenol Analogue Exposure on Mouse Adipose Gene and Protein Profiles

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Bisphenol A (BPA), reported to be an endocrine disrupting compound (EDC), is a high production volume chemical used in the manufacturing of plastics and epoxy resins. BPA has been associated with aberrant reproductive and developmental effects in rodents and humans, including obesity and metabolic disease. A recent voluntary replacement of BPA with other bisphenolic chemicals, including the fluorinated and sulfonated derivatives, BP4F and BPS, leaves many research gaps regarding the effects of these bisphenol analogues on adipose tissue. These studies assessed the effects of BP4F and BPS prenatal exposure on adipose tissue gene and protein expression in mice; and adipogenesis, lipid accumulation and gene expression in murine 3T3-L1 preadipocytes in culture. Timed pregnant CD-1 mice were exposed to vehicle, BP4F (5mg/kg), or BPS (0.5mg/kg) via oral gavage between gestation days 10–17. White adipose tissues were collected and processed from resulting offspring at postnatal day (PND) 35 and 90 for RNA and protein evaluation. Quantitative real-time PCR (qPCR) data revealed increased mRNA levels of several genes in the prenatally BPS exposed females on PND35; including Pparγ, Gpr30, and Esr2. PND35 BPS males exhibited increased levels of Esr2, and decreased Gpr30 and Esr2 expression. PND35 BPS male mice exhibited increases in both Pparγ and Esr1. At PND90, we observed more changes in adipose gene expression in BPS females, including increased levels of Cebpa, Fasn, Acaca, Acacb, Esr1 and decreased Gpr30 and Esr2. PND90 BPS males exhibited increased Fasn, Lpl, Acaca, Gr, and Esr2. Prenatal BPS female exposed mice and males at PND90 showed little to no change in gene expression compared to vehicle control mice. Preliminary protein data showed elevated FAS and FABP4 levels in BPS exposed females at PND90. Also, 3T3-L1 preadipocyte cells were induced to differentiate and treated in parallel with BP4F, BPS, BPAF, and BPS (serial log dilutions of 1µM to 10µM) for 8 days. High-content imaging was used to analyze adipocyte number and lipid droplet accumulation. RNA was isolated on day 8 for qPCR analysis. High-content imaging of adipocyte cells treated with bisphenolic chemicals resulted in significant increases in both lipid accumulation and adipocyte number (BPS>BP4F>BPSA). qPCR analysis of adipsigenic genes on day 8 of exposure to these chemicals are on-going. These data appear to be consistent with the growing evidence that EDCs, like bisphenol analogues, have the potential to disrupt adipogenesis and human health.

2408 In Vitro and In Vivo Evaluation of Bisphenol Analogue Exposure on Mouse Adipose Gene and Protein Profiles

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2411 In Utero and Lactational (IUL) TCDD Exposure Impedes Urethral Urinary Flow in Male Mice


In utero and lactational (IUL) 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure exacerbates anastomotic dysfunction in mouse model that mimics urinary symptoms in men with benign prostatic hyperplasia. These symptoms include incomplete bladder emptying and increased urinary frequency linked to impaired urethral urethral passage. In male mice exposed to IUL or vehicle TCDD we used a urine void spot assay to assess urination frequency, contrast-enhanced ultrasound to determine velocity of urethral passage, and an α-adrenergic receptor antagonist to relax prostate smooth muscle and reduce urethral urinary flow. We hypothesized that IUL TCDD exposure would increase the frequency of small volume voids and the velocity of urethral passage (indicators of prostate-induced urethral obstruction). We also hypothesized an α-adrenergic receptor antagonist would reduce urinary flow. Prostate-specific C57Bl/6J dams were gavaged with TCDD (1 µg/kg maternal dose) or corn oil (5 mL/kg vehicle control) at embryonic day 13. Male pups were aged 6 weeks and given either subcutaneous slow release implants of testosterone (25 mg) and estradiol (2.5 mg) to induce urinary dysfunction or a sham operation (controls). Urinary function was assessed biweekly using the non-invasive void spot assay. At 8 weeks, mice were anesthetized, DEFINITELY contrast agent was injected into the bladder, and ultrasound was used to visualize urethral passage of contrast agent. This velocity was recorded at baseline and 1, 5, and 15 min after treatment with an α-adrenergic receptor antagonist (Tamsulosin, 100 µg/kg iv). Mice treated with hormones for 2 weeks showed more voids in smaller volume spots than sham operated controls, evidence of increased urinary frequency. IUL TCDD treatment combined with hormone implantation appeared to most dramatically increase urinary frequency compared to all groups, while IUL TCDD treatment without hormone implantation did not change urinary frequency compared to untreated controls. Velocity of contrast agent passage through the urethra was greater in IUL TCDD + hormone treated mice than in corn oil + hormone treated mice, indicating urethral obstruction. Tamsulosin decreased contrast agent velocity in all groups, suggesting it lessened obstruction, but the change was greatest in IUL TCDD treated animals. These results provide compelling evidence that IUL TCDD exposure + hormone treatment increases prostate smooth muscle tone to reduce urethral urine passage. Funded by RO1ES001332, F31ES028594, US4DK104310, T32 ES007015.

2412 Maternal Exposure to Polynbrominated Diphenyl Ether (PBDE47) Increased Asthma Susceptibility in Adult Offspring


Polynbrominated diphenyl ethers (PBDEs) found in flame-retardants can easily transfer to the fetus through the placenta and be transferable in breast milk. Studies have linked PBDEs to cognitive dysfunction and inflammatory responses. However, there has been no evidence that PBDEs can cause asthma in offspring. We hypothesized that PBDE47 (the most common congeners of PBDEs) was associated with tumor necrosis factor-alpha (TNFα) promoter methylation and protein expression in female cord blood. In this study, we hypothesize maternal PBDE47 exposure could modulate offspring’s inflammatory responses, perhaps through epigenetic regulation of gene transcription. Objective: We examined the effect of maternal exposure of PBDE47 on female offspring’s asthma risk using an experimental asthma model. Methods: We exposed female adult B6/S129 mice to 0.1mg/mg/kg bw of PBDE47 or vehicle control (corn oil) at pre-conception, embryonic days 7 and 9, and postnatal day 4 and 16 via oral gavage. Six-week-old offspring were challenged with 100µg of house allergen (house-dust-mites, HDH) s.c. v/v per day. Tmajuant C57Bl/6J dams were gavaged with HDH-induced AHR. We also assessed the airway inflammation by examining immune cells profile in bronchoalveolar lavage (BALF) and IgE/G production in blood plasma. Moreover, we performed reduced represented bisulfite sequencing (RRBS) to identify differential DNA methylation changes in mouse lung. Results: Female offspring from PBDE47-exposed mother showed a lower airway sensitivity compared to those from unexposed mother. Maternal exposure to PBDE47 did not modulate the induction of eosinophils and IgE/G production in response to HDH challenges. Strikingly, RRBS data revealed PBDE47-mediated differential DNA methylation patterns in offspring’s lung tissues. Gene candidates enriched in cytokine signaling, Wnt signaling and integrin signaling pathways that may contribute to cell proliferation, contraction and cytokine production in lungs. Future studies are required to validate the role of these signaling pathways on asthma pathogenesis. Herein, our data suggest maternal exposure to PBDE47 increased offspring’s asthma risk. This response, independent of airway inflammation, may be driven by epigenetic alterations in modulating DNA methylation of genes regulating structure and function in the lung.

2413 Peri-implantation Ozone Exposure Induces Sexually Dimorphic Placental-Fetal Brain Axis Abnormalities


Peri-implantation ozone exposure induces fetal growth restriction in the rat. However, the influence of placental health in mediating the impact of ozone on fetal size and neurodevelopment has not been studied. Therefore, we examined placental metabolic adaptations and alterations in neurodevelopment. To this end, we exposed pregnant female Sprague-Dawley rats to ozone (2.63 ± 0.05 ppm) 1 hour/day starting on gestation day (GD) 19 from Long-Evans rats, we investigated placental metabolic endpoints including oxygen consumption and respiration, mitochondrial biogenesis, and changes in macronutrient concentrations and markers of autophagy. Fetal energy homeostasis was determined using body composition, hepatic triglycerides, and hypothalamic RNAseq. Results show that male placentas from ozone-exposed dams had increased mitochondrial respiration, ATP production, increased mitochondrial content, and reduced concentrations of triglycerides, cholesterol, and glucose. While female placentas failed to show these changes, they had evidence of AMPK phosphorylation and autophagy. Taken together, we next hypothesized that male and female fetuses would have sex-specific differences in body adiposity related to the aforementioned placental adaptations. Accordingly, male fetuses from ozone-exposed dams had reduced adiposity and hepatic triglyceride levels, whereas the female fetuses did not. Lastly, RNAseq within the hypothalamus of male fetuses revealed a reduction in ribosomal protein mRNA, a phenomenon that may be related to reduced amino acid availability for protein diminished cellular growth. While the hypothalamus of female fetuses did not appear to be nutrient-starved, RNAseq analysis of the hypothalamus revealed significant reductions in serotoninergic and dopaminergic genes. Furthermore, female hypothalami had reduced autophagic-related gene expression and increased ribosomal protein mRNAs. Together, we demonstrate that following peri-implantation ozone exposure, the placenta adapts in a sexually dimorphic manner. This adaptation may impact the nutrient availability of the fetus and disrupt neurodevelopment, rendering offspring sensitive to neurological deficits later in life. Disclaimer: This abstract does not reflect US EPA policy.

2414 Gestational Exposure to Inhaled Nano-Sized Titanium Dioxide Impairs Fetal Nutrition


Exposure to engineered nanomaterials (ENM) during pregnancy has the potential to alter both the mother and fetus through a variety of host behavioral, physiological and metabolic mechanisms. The molecular mechanisms associated with impaired fetal growth remains unknown. To investigate nutritional impairments associated with ENM exposure, 6 pregnant Sprague-Dawley rats were exposed to nano-sized titanium dioxide (nano-TiO2) aerosols beginning at gestation day 4 (9.35 ± 0.15 µm, m, 11 days, 5 hours/day, mean particle size 162 ± 7.67 nm) (HPGA, IESTechno) and continued through GD 19. Comparisons were made to 6 GD 20 Naive (un-exposed) control rats. Litter characteristics, maternal, and fetal plasma were collected on GD 20. ENM exposed animals had smaller litter sizes (15.7 ± 0.6 control pups per litter vs. 11.0 ± 0.6 exposed), placental weights (0.54 ± 0.01 g control placenta vs. 0.216 ± 0.01 g exposed placenta) (410.5 ± 11.2 g control dams vs. 339.3 ± 14.5 g exposed dams), and maternal fetal plasma was assessed for ω-3 and ω-6 PUFA concentrations using liquid chromatography-mass spectrometry (LC-MS) techniques. In these, palmitic acid was lower (p=0.053) in the exposed (2.21 ± 0.11 µg/mL) fetal plasma compared to control (2.67 ± 0.10 µg/mL). Further, PUFAs placental transport mRNA expression of fatty acid uptake (lipoprotein lipase (0.35 ±0.12)), trafficking (FABPA (0.80 ±0.42)), and synthesis (Elovl (0.45 ±0.08)) genes were significantly reduced, as normalized to control, after a single 5-hour nano-TiO2 inhalation exposure. These results indicate that exposure to ENM aerosols during gestation may affect placental fatty acid transport, synthesis and utilization, leading to impaired fetal growth and IUGR phenotype. Future directions will explore maternal PUFFA supplementation to improve health outcomes and promote fetal development. Supported by NIH-R00-ES024728; P30-ES050522; T32-ES007148; R25-ES052072; ASPET-SURF.
2415 Alterations in Fetal Lung Development by Maternal Allergen and Methyl Enriched Diet Exposure

Early life exposure to allergens is a risk factor for the development of asthma in later life. Furthermore, the effects of maternal-fetal interaction on offspring asthma susceptibility remain understudied, even though pregnant women are recommended to increase folate intake during pregnancy. Here, we aim to determine the joint effect of maternal exposure to allergens and methyl enriched diet on offspring lung development. Methods: Female C57BL/6 mice were maintained on either regular chow or methyl enriched (ME) diet before conception and until harvest. They were sensitized to house dust mite (HDM), a ubiquitous indoor allergen, two weeks before mating. Dams were further challenged to HDM three times a week until harvest at embryonic day 14. Unexposed dams were administered saline as a control. We measured placental weight, pup sex, and sex ratios in both groups. To determine sex to exposure to HDM and ME diet on fetal growth outcomes. We also examined the expression of genes related to fetal lung growth in female pups. We showed that maternal exposure to ME diet, with and without HDM exposure, significantly increased pup weight (P<0.001) and length (P<0.001). ME diet alone did not change expression of dihydrofolate reductase (Dhfr), but in combination with maternal HDM exposure, female pups displayed reduced mRNA levels of Dhfr in fetal lungs when compared to those from unexposed dams (P<0.05), or HDM-exposed dams on regular chow (P<0.005). In addition, maternal ME diet was associated with a significant increase in corticotropin-releasing hormone (Crh) mRNA levels in fetal lungs compared to pups from dams fed regular chow (P<0.01). Also, pups of HDM-exposed dams fed ME diet displayed increased Crh expression when compared to pups from HDM-exposed dams fed on regular chow (P<0.05). Conclusion: ME diet is rich in methyl donors, suggesting a role in epigenetic regulation of gene transcription. In addition to key roles in folate metabolism, Dhfr can regenerate tetrahydrobiopterin (BH4) from dihydrobiopterin (BH2), thus coupling eNOS, which is known to be critical for lung development. Upregulation of Crh by maternal ME diet intake is intriguing, particularly given that fetal expression of Crh in lungs is crucial for the maturation of type II alveolar cells, Club cell maturation, and surfactant synthesis. Taken together, our data suggest a joint effect of maternal exposure to HDM and ME diet on fetal lung development, which may modulate asthma risk in later life.

2416 Rats Exposed to Nanoparticles during Gestation Do Not Develop Metabolic Disease in Early Adulthood
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Disruption of the maternal environment during gestation can impact fetal health leading to intrauterine growth restriction, low birth weight, and risk of cardiovascular/metabolic disease in adulthood. In our model of maternal in-halation of engineered nanomaterials (ENM) during pregnancy, we have identified hemodynamic perturbations in the maternal, fetal, and offspring vasculature after chronic exposure initiated on gestational day (GD) 4. However, neither the cardiovascular implications of late-stage gestational exposures, nor the cardiometabolic effects in the progeny past 12-weeks of age are known. Timed-pregnant Sprague-Dawley rats were exposed to nano-sized titanium dioxide aerosols (9.35 ± 0.15 mg/m³), 4 hours, primary particle size 21 nm, median particle size 162 ± 7.67 nm (SMPS, TSI)) 5 days per week via whole-body inhalation (HPGA, IESTechco) starting on GD 4 or 17, prior to delivery (GD 20). A subset of animals was exposed to filtered air during gestation as controls. Animals delivered in-house and offspring were weighed weekly. At 6 months, a cohort of male and female progeny were exposed to a chronic fat- and fat-free mass [computed tomography (CT)] along with fasted plasma concentrations of glucose, total cholesterol, LDL, HDL (Pointe Scientific), and insulin (Sigma-Aldrich). At 6 months, we identified progeny derived from exposure to ENM during pregnancy to assess the effects of maternal-fetal stress, and to determine the influence of maternal environments on sex-specific cardiometabolic disease in the progeny in early adulthood. Further analyses will be conducted to evaluate mechanisms of maternal-fetal stress, inflammation, oxidative stress, epigenetics, and lipoproteins to identify the molecular link between maternal exposure and offspring health. Supported by NIH-R00-ES024783; P30-ES005022; ASPET-SURF.

2417 The Sex-Specific Influence of Gestational Air Pollution Exposure on Placental Metabolic Gene Expression within the Rhode Island Child Health Study (RICHIS)
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Morbidity and mortality due to air pollution is a growing burden to society. Studies have demonstrated that exposure to ambient PM2.5 contributes to adverse metabolic health outcomes. Given the importance of the placenta in determining fetal growth and its potential to influence metabolic health outcomes in later life, we explored the influence of PM2.5 exposure on sex-specific placental metabolism within the Rhode Island Child Health Study (RICHIS) cohort population. Using Gene Ontology annotations, a list of placental metabolic genes (n=657) was constructed related to lipid and/or glucose metabolism. This list of genes was then curated to include genes expressed in the term placenta (n=657) via the Gene Ontology database and placental RNA-seq data (n=148) from RICHIS, which was used to assess expression of the selected genes. Linear regression analyses were employed to determine the association between placental metabolic gene expression and PM2.5 exposure in a sex-specific manner. Thirty-two genes were significantly associated with PM2.5 exposure, out of which 24 genes were associated in a sex-specific manner. We selected 5 genes for RT-PCR verification in additional placentals from RICHIS, which included 2 significant genes from each sex-specific analysis and one gene (CLTCL1) that was significantly associated in both sex-specific analyses. CLTCL1 was upregulated in male term placentas, but downregulated in female term placentas. Placental metabolic genes associated with PM2.5 identified in this subset are involved with lipid rather than glucose metabolism. Funding: NIH-NIEHS P30ES023515.

2418 Sex-Dependent Effects of Early-Life Cadmium Exposure and High-Fat Diet on the Liver, Heart, and Kidney
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More than 1/3 of adults in the United States are considered obese. Obesity is a primary risk factor for a spectrum of inflammatory disease, non-alcoholic fatty liver disease and cardiometabolic syndrome. Other genetic and/or environmental factors however contribute to their pathogenesis. Data suggest that exposure to the environmental metal toxicant cadmium (Cd) is a risk factor for these metabolic diseases. Additionally, gender may be a contributing factor. To explore the relationship among obesity, Cd exposure and gender C57BL/6J mice were exposed to 0, 0.5 or 5 ppm Cd-containing drinking water for >2 weeks before breeding. Pregnant dams and offspring were then continuously exposed to the same Cd concentration. At weaning, offspring were fed either low-fat (LFD) or high-fat (HFD) diets. Animals were sacrificed PWN 10 or 24 and liver, kidney and heart collected for histopathological and biochemical analyses. Female mice accumulated significantly more Cd in liver and kidney, but not heart, compared to males. In the heart, only female mice developed cardiac fibrosis with the combination of HFD and Cd exposure (Sirius Red staining, increased collagen protein and mRNA expression). In the liver, Cd exposure exacerbated HFD-induced liver injury in male, but not female mice (histology and total cholesterol levels). Although more Cd accumulated in female kidneys, Cd-exacerbated, HFD-induced changes in renal parenchyma were significantly greater in male mice. In addition, proteomic analysis identified a number of sex-dependent differences in canonical renal signaling pathways. These results indicate gender-specific responses to Cd exposure in these organs that cannot solely be attributed to difference in Cd accumulation. Future work will focus on elucidating molecular mechanisms responsible for the observed sexual dimorphic phenotypes.
Long-Lasting Effects of Developmental Exposure to 2,2',4,4'-Tetramethylphenyl Ether on Blood-Liver Balance of Lipids in Male Mice

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2,2',4,4'-Tetramethylphenyl ether (BDE-47) is one of the dominant congeners of polybrominated diphenyl ethers (PBDE) - a group of flame retardants that have been widely used in North America and Europe over the last 50 years until recent industrial ban. Around 20% of U.S. population was born during the maximum plateau of PBDE environmental concentration. PBDEs are strongly resistant to degradation and persist in environment. The study was designed to analyze long lasting metabolic effects of exposures to BDE-47. CD-1 mice were prenatally or neonatally exposed to 1 mg/kg body weight of BDE-47 or vehicle, and changes in liver histology, transcriptome, and liver-blood balance of triglycerides were monitored in 10 month old male offspring. In both exposure groups, long term reprogramming of lipid metabolism was clear, including increased liver triglycerides and decreased blood triglycerides, and altered expression of metabolic genes in the liver. Significant up-regulation of lipid influx transporter Cd36 in pre- and neonatal exposure groups was seen as a potential mechanism of blood/liver imbalance of triglycerides. Opposite directional changes in expression of many metabolic genes including Cd36 and levels of circulating triglycerides were observed in comparison with our previous studies of transcriptomic signature of PBDE, which used lower exposure dose (0.2 mg/kg body weight). In conclusion, this study shows that environmentally relevant developmental exposures to BDE-47 permanently alter blood-liver balance of lipids, with low and moderate doses having opposite effect on liver transcriptomics and triglyceride balance. These results suggest that PBDE exposure may be an important factor associated with heath conditions, such as risks of cardio-vascular diseases and non-alcoholic fatty liver via modulation of blood/liver balance of lipids. Further studies for these findings are needed to be relevant to human diseases.

Early-Life TCDD Exposure Shapes Gene Expression across the Life Course of Mice

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a potent environmental toxin that is generated as a byproduct of industrial operations involving high temperature processing of organic material. It enters into environmental systems as a constituent of solid waste and flue gas. In vertebrate systems, TCDD activates the AhR-mediated xenobiotic response which modulates transcription of numerous genes responsible for metabolizing toxic compounds. The World Health Organization recognizes links between early-life exposure to TCDD and late-onset pathologies including neurological disability, reproductive impairment, and increased cancer risk. Our goal is to understand the consequences of early-life TCDD exposure on the molecular state of multiple tissues. Mice were exposed to TCDD from preconception through gestation and lactation. Tissue samples were taken three weeks, five weeks, twenty weeks, and forty weeks after birth. From our measurements of transcriptional profiles, we show that gestational TCDD exposure shapes gene expression both in the short-term and in the long-term. Furthermore, we performed analyses of open chromatin using ATAC-seq to contrast chromatin accessibility with associated gene expression. Early-life exposure to TCDD resulted in substantial changes in gene expression after accounting for tissue, age, and sex. A total of 2493 genes were differentially expressed in liver combined over both sexes and ages. Blood showed few differentially expressed genes at three weeks but substantially more in adult mice. The effects of TCDD differed dramatically between males and females. Furthermore, there is no overlap between the response in liver and in blood. Though there were clear gene expression signatures of TCDD exposure at both ages, the changes observed at three weeks did not persist into adulthood. We conclude that a complex cascade of gene regulatory events are set in motion by early-life TCDD exposure that result in long-term gene expression differences in adult mice.

Developmental Exposure to Brominated Flame Retardant 2,2',4,4'-Tetramethylphenyl Ether (BDE-47) Permanently Reprograms Liver Lipid Metabolism in Mice via mTOR-Dependent Pathway

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Abnormally high accumulation of triglycerides (TGs) in hepatocytes is called hepatic steatosis or non-alcoholic fatty liver disease (NAFLD). It is the most common chronic liver disease among adults and children, with 33% to 88% prevalence. NAFLD increases the risk of type 2 diabetes and many other morbidities. Increased blood triglyceride levels result in atherosclerosis, a major cause of heart attack. An emerging hypothesis connects the metabolic disease epidemic with chemical exposures during vulnerable windows of early development. Evidence suggests that developmental exposure to flame retardant BDE-47 permanently reprograms lipid metabolism, resulting in a NAFLD-like phenotype. These changes were associated with changes in activity of the mTOR pathway, an intracellular pathway that regulates aspects of the cell cycle and metabolism. Previously, we showed that BDE-47 activates mTOR signaling in mouse livers and permanently alters expression of fatty acid translocase Cd36, a membrane receptor responsible for uptake of fatty acids in hepatocytes. This discovery and network analysis identified several pathways that were differentially expressed. Key pathways impacted included oxidative stress, mitochondrial dysfunction, and fatty acid metabolism. These results show that Cd exposure during gestation specifically affects liver function and increased weight in female mice. Ongoing functional genomic studies will further explore the nature of the sexually dimorphic responses to Cd exposure and examine the ontogeny of the Cd-induced changes in hepatic transcriptome prior to postnatal day 42 in the mouse liver. Potential epigenetic mechanisms driving Cd-induced metabolic changes, including differential methylation of metal ion transporter promoter regions, are being explored.
changes in Cd36 expression and changes in triglycerides in blood. These results may provide relevant mechanistic information critical to understanding and addressing the environmental factors involved in current epidemic of NAFLD and atherosclerosis.

2423 Reprogramming of Peroxisome Proliferator-Activated Receptor Target Genes in Mice Perinatally Exposed to Phthalates

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Phthalates have been demonstrated to activate peroxisome proliferator-activated receptors (PPARs), nuclear receptors that act as transcription factors for a plethora of target genes involved in metabolism. Developmental exposures to phthalates have been linked with adverse metabolic outcomes in animal and human studies, but underlying mechanisms remain unclear. We investigated reprogramming of PPAR target genes as a link between perinatal phthalate exposures and later-in-life metabolic outcomes in an established mouse model of human-relevant exposure. Dams were exposed to phthalates via phytoestrogen-free diet from 2 weeks prior to mating until weaning at postnatal day 21 (PND21). Dams were randomized to one of four experimental groups: 1) 7% corn oil control chow, 2) 25mg DEHP/kg chow, 3) 75mg DINP/kg chow, or 4) 25mg DEHP + 75mg DINP/kg chow. One male and female offspring per litter were weaned onto control chow and followed to 10 months of age. Histological analysis of tissues from sacrificed embryos and postnatal day 21 (PND21) sequencing was performed on RNA isolated from livers collected at PND21 and 10 months (n=6/sex/group/age). Differential expression analysis was carried out via DESeq2 comparing each group to controls, stratifying by age and sex. The group with the greatest number of differentially expressed genes in the liver (adjusted p<0.10) was females perinatally exposed to DINP only, with 64 genes at PND21 and 175 genes at 10 months of age. To identify targets in the liver that were reprogrammed by perinatal phthalate exposures, we examined pathway enrichment for genes that were differentially expressed at both PND21 and 10 months via LRPath. Females perinatally exposed to DINP-only had the largest number of significantly enriched KEGG pathways (23 pathways with FDR<0.05). Enriched pathways included biosynthesis of unsaturated fatty acids (FDR=0.010), PPAR signaling pathway (FDR=0.042), fat digestion and absorption (FDR=0.042), and type II diabetes (FDR=0.047). Genes driving these metabolic pathways included several PPAR target genes, indicating that they may be reprogrammed in the liver by perinatal exposure to DINP, particularly in females. Ongoing work to quantitatively validate expression and investigate DNA methylation at these candidate reprogrammed PPAR target genes is underway, as well as parallel analyses in adipose.

2423a Participation of Endothelial Small GTPase RhoA in Embryo Development and Retinal Angiogenesis

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Angiogenesis, the formation of new blood vessels, is a highly orchestrated process requiring a well-regulated balance between pro-angiogenic and anti-angiogenic factors and corresponding signaling networks. Imbalanced angiogenesis is a characteristic of several diseases such as cancer, inflammation, rheumatoid arthritis, atherosclerosis and age-related macular degeneration. The Rho family of small GTPases plays a central role in angiogenesis, signal transduction, gene expression which are necessary for vascular development and angiogenesis. Among the small GTPases of the Rho family, RhoA, Rac1 and Cdc42 are the best characterized. Although the role of endothelial Rac1 and Cdc42 in embryonic vascular development and retinal angiogenesis has been studied recently, the role of endothelial RhoA is yet to be explored. In this study, we aim to identify the role of endothelial RhoA in vascular development in vivo by generating endothelial RhoA deficiency through the Tie2-Cre and Cdhs-CreERT2 promoters. In vivo deletion of RhoA in embryonic endothelial cells leads to decreased survival of endothelial RhoA deficient mice. On the other hand, inducible RhoA deficiency in the retinal vessels in different developmental days did not affect the radial growth, the number of filopodia per area and the area with deep vascular plexus. In vitro, RhoA seems to be involved in endothelial cell migration, invasion and sprouting triggered by important angiogenesis inducers, such as Vascular Endothelial Growth Factor (VEGF) and Sphinogistine-1 Phosphate (SIP). These ongoing experiments aim to provide a better understanding of the numerous functions regulated by the endothelial small GTPase RhoA in normal physiology and human diseases.

2424 Shipping Study to Evaluate the Performance of the Labcyte Epi-Model 24 Tissues for Use in the Skin Irritation Test (OECD Test Guideline 439) after Long-Haul Airfreight

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It is recommended that an evaluation of the impact of shipping Reconstructed human Epidermis (RHE) tissues be conducted especially after long-haul airfreight shipments. The OECD Test Guideline 439, in vitro Skin Irritation: Reconstructed Human Epidermis Test Method, recommends that users do so by verifying the barrier properties of the tissues after receipt. In this study, LabCyte EPI-MODEL 24 tissues were received in the USA after an overnight shipment from Japan and were tested to evaluate their performance after shipment using several endpoints. First, the viability of untreated tissues incubated overnight in culture medium was assessed using the vital dye MTT and expressed as Optical Density (OD) values. The calculated OD values were 1.2 (JTEC) and 0.99 (IVS) and within the range established by the manufacturer (0.8 – 2.5). The barrier function was further evaluated after the tissues were exposed to the assay negative control (Phosphate Buffered Saline - PBS) and to four concentrations of the positive control, Sodium Lauryl Sulfate (SLS) (1.0, 2.0, 3.0 and 4.0 mg/mL). The calculated IC50 values were 2.7 mg/mL (JTEC) and 3.4 mg/mL (IVS), respectively, and within the established historical range (1.4 - 4.0 mg/mL). The analysis of the results generated by the two labs (JTEC and IVS) demonstrated that the tissue lot met the acceptance criteria developed by the tissue manufacturer under conditions of shipping stress. Finally, the histological analysis of untreated fixed tissues identified all tissue layers and supported the conclusion that the tissue model was acceptable for use in subsequent studies after long-haul airfreight shipment. Such shipping studies are critical to gaining confidence in the tissues’ performance when used for research, industrial, and regulatory testing purposes.

2425 Increasing Confidence in the EASA Skin Sensitization Assay Using a Measurement Science Approach

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NICEATM is currently coordinating a multi-laboratory validation study to characterize the usefulness and limitations of the Electrophilic Allergen Screening Assay (EASA) for risk and hazard assessment for skin sensitization classification. The EASA assay functions by assessing the ability of potential skin sensitizer compounds to elicit a chemical reaction by assessing depletion of one of two probe molecules using absorbance or fluorescence measurements. However, the use of this assay in a single cuvette format resulted in multiple measurement challenges such as low throughput, which hindered the ability to include additional control measurements, and a lack of access to laboratories still running single cuvette assays. To overcome these limitations, it was decided to redesign this assay protocol to work with a 96-well plate format. Cause-and-effect (C&E) analysis was performed to identify key sources of variability and potential biases which could impact the test results. Based on the C&E analysis, process control measurements were incorporated into a 96-well format to quantify key sources of variability each time the assay is performed. Additional control experiments to evaluate edge effects, cross-talk, well-to-well variability and statistical power of the assay system were also performed during system characterization. Ten compounds from Phase 1 of the inter-laboratory EASA study that were previously tested in the single cuvette assay, and 5 additional compounds were tested in the 96-well plate design. One key insight revealed by this process was the interference from five of the original ten Phase 1 test compounds (solubilized in acetonitrile) which would not have been previously detected using the single cuvette assay. Not taking the interference of these 5 compounds into account has been shown to lead to potential false negative and false positive identifications. Overall, the approach described here provides steps that can be taken to increase confidence in the EASA assay and serve as a platform for inter-laboratory comparisons, as well as reducing unseen bias which may have existed in previous assay development.

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**2427** The Dermal Sensitization Threshold (DST)
Approach for Botanical Extracts Evaluated as Negative in *In Vitro* Test Methods; Botanical DST


Cosmetic ingredients often comprise naturally derived complex mixtures, such as botanical extracts, which may contain skin sensitizing constituents. For skin sensitization assessment methods of botanical extracts without animal testing, a strategy using non-target analytical screening techniques has been proposed. However, non-target qualitative and quantitative screening analyses of skin-sensitizing constituents in naturally derived complex mixtures are often difficult and resource-intensive. To address this problem, we developed a risk assessment strategy for undetermined constituents of botanical extracts by combining exposure assessment in addition to the hazard identification using the binary test battery with KeratinoSens® and h-CLAT. In our previous study for the sensitivity of the evaluations of skin sensitizing constituents in botanical extracts using the binary *in vitro* test battery with KeratinoSens® and h-CLAT, some sensitizers showed higher detection limits in *in vitro* test methods than in murine local lymph node assays (LLNA). Thus, to minimize the uncertainty associated with decreased sensitivity for these sensitizers, a risk assessment strategy was developed for botanical extracts with negative results from the binary test battery. Assuming that the no expected sensitization induction level of botanical extracts (botanical NESIL) can be derived for botanical extracts with negative *in vitro* test results, we assessed 146 sensitizers with *in vitro* and LLNA data according to the assumption of indeterminate constituents in botanical extracts. Next, we calculated 95th percentile probabilities of botanical NESILs and derived 6,010 μg/cm² as a dermal sensitization threshold for botanical extracts (botanical DST) with negative *in vitro* test results. Finally, we performed feasibility studies of this botanical DST value as an acceptable formulation ratio of botanical extract evaluated as negative in the binary test battery and performed the botanical DST approach for practical product examples. Feasibility studies indicated that acceptable formulation ratios of botanical extract evaluated as negative in the binary test battery were calculated as > 100% for rinse-off products and 0.3% - 12% for leave-on products. This approach would be a novel risk assessment strategy for incorporating the DST approach and information from *in vitro* test methods.

**2428** U-SENS: New Perspective for Chemicals Interfering with Fluorescence by Flow Cytometry

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The U937 Cell Line Activation Test U-SENS™ is one of the *in vitro* OECD endorsed test method (TG442E) along the skin sensitization AOP (Adverse Outcome Pathway). It could be used for hazard identification, potency and risk assessment. Experimental procedure: the human myeloid U937 cell line (CRL-1593.2) was treated for 45 h with a dose-range of substances. The CD86 (B70/ B7-2) costimulatory molecule expression was then measured by flow cytometry using the FITC mouse anti-human CD86 monoclonal antibody. Propidium iodide was used as the viability markers to exclude dead cells from the analysis. Some substances such as well-known hair dyes increase the auto-fluorescence of cells in the FITC channel by flow cytometry compromising the quantification of the CD86 induction. To avoid such interferences and biases, an alternative of the fluorophore FITC (excited at 488 nm), the APC (excited at 633 nm) has been evaluated. Based on the study of 4 interfering substances (1,4-phenylenediamine (pPD), 2-Methyl-p-phenylenediamine sulfate salt (pTD), 2,4,5,6-Tetraaminopyrimidine Sulfate (TAP), and 1,3-Phenylenediamine (mPD)), the non-specific fluorescence increase was drastically reduced with APC leading to clear dose response curves. The approach using an APC-coupled mouse anti-human CD86 monoclonal antibody was further validated on a set of 24 substances composed of an equal number of sensitizers and non-sensitizers. All acceptance criteria applied to the standard protocol were met (CV<0.7, EC150, CD86 baseline expression, ...). As a conclusion, the use of the alternative APC coupled antibody opens new perspectives to evaluate skin sensitizers interfering with the fluorescence at 488 nm in the U-SENS™ test method (OECD 442E).

**2429** The Kinetic Direct Peptide Reactivity Assay (k-DPRA): An *In Chemico* Method to Characterize the Skin Sensitization Potency of Chemicals

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While the skin sensitization hazard of substances can meanwhile be identified using non-animal methods, the classification of potency UN GHS sub-categories 1A and 1B remains challenging. The kinetic DPRA (k-DPRA) is a modification of the DRPA (OECD TG 442C). In the k-DPRA the reaction kinetics of a test substance towards a synthetic cysteine-containing peptide is evaluated. For this purpose, several concentrations of the test substance are incubated with the synthetic peptide for several incubation times at 25°C. After the respective incubation time, the reaction is stopped by addition of the fluorescent dye monobromobimane. The highly reactive and non-fluorescent monobromobimane rapidly reacts with unbound cysteine moieties of the model peptide to form a fluorescent complex. The remaining non-depleated peptide concentration is determined thereafter by fluorescence measurement. By stopping the reaction with monobromobimane, the extent of peptide depletion can be determined at precisely defined time points. Kinetic rates of peptide cysteine-depletion are then used to distinguish between two levels of skin sensitization potency, i.e. to discriminate between CLP/UN GHS sub-categories 1A and 1B. In addition, kinetic rates generated with this method have a strong quantitative correlation to sensitizer potency and can therefore be used in defined approaches (DA) with a quantitative data integration procedure (DIP) for skin sensitization potency assessment. During an in house validation (Wareing et al., 2017) 35 of 38 substances with known sensitization potency were correctly assigned to the potency sub-categories (compared to LLNA data), and the predictivity for 14 human data was equally high. These results warrant the k-DPRA for further validation and that it be used as part of defined approach(es) to assess skin sensitization in order to fully replace *in vivo* testing for assessing skin sensitization including potency sub-classification. A multi-laboratory validation study is ongoing.
2430 A New Method for Skin Sensitization Potential for Poorly Soluble Compounds Using a 3D Keratinocyte / THP-1 Co-Culture

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Allergic contact dermatitis is caused by skin contact with an allergen. The identification and assessment of the sensitising potential of new compounds is essential to prevent allergic response. The development of alternative methods is required for the cosmetics industry considering the questionable relevance of the use of animal testing to predict human hazard and the effective ban on the use of animals for safety testing. For now, a combination of different tests is required to predict the skin sensitising potential of chemical substances, each providing information on one or more key steps of the mechanism. Among these assays, six tests are already validated by European Centre for the Validation of Alternative Methods (ECVAM) and test guidelines are edited by OECD, namely Direct Peptide Reactivity Assay (DPRA), KeratinSensTM, LuSens, human Cell Line Activation Test (h-CLAT), U937 cell line activation test (U-SENS®) and the interleukine-8 reporter gene assay (IL-8 Luc assay). To circumvent the solubility limitation of the tested compound in the ECVAM validated methods and in particular in h-CLAT protocol, a new in vitro method is designed using a co-culture model. We developed a co-culture system consisting of VitroDerm, an in-house reconstructed human epidermis (RHe) and a monocyctic cell line (THP-1) and the optimisation of the h-CLAT protocol has been performed. A comparison between regular h-CLAT (THP-1 monoculture) and h-CLAT using the VitroDerm / THP-1 co-culture is carried out on 12 reference chemicals (sensitisers or non sensitisers). CD86 and CD54 were quantified by flow cytometry on THP-1 cells. The VitroDerm / THP-1 co-culture model allows a correct classification of 12 reference chemicals compared to THP-1 monoculture leading to an accuracy, specificity and sensitivity of 100%. The major advantages of this method is to take into account the bioavailability factor with skin metabolism aspects. This test can be used to assess skin sensitization potential of poorly soluble compounds. Finally, the VitroDerm / THP-1 co-culture opens new horizon on intercellular interplay via paracrine interaction.

2431 Skin Sensitization Potential: Addressing the 3 Key Events of AOP Using a 3D Keratinocyte / THP-1 Co-Culture

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Allergic contact dermatitis is caused by skin contact with an allergen. The identification and assessment of the sensitising potential of new compounds is essential to prevent allergic response. The development of alternative methods is required for the cosmetics industry considering the questionable relevance of the use of animal testing to predict human hazard and the effective ban on the use of animals for safety testing. We developed a co-culture system consisting of VitroDerm, an in-house reconstructed human epidermis (RHe) and a monocyctic cell line (THP-1) to use this in the context of skin sensitisation analysis. Whilst the methods already validated by ECVAM address one key event, the VitroDerm / THP-1 co-culture enables the modelling of the first three key events of the skin sensitisation Adverse Outcome Pathway (AOP): the covalent binding of a chemical to cutaneous proteins (key event 1), the activation of keratinocytes (key event 2) and the dendritic cell activation (key event 3). First, the co-culture model was characterised (cell viability, histology and basal expression of CD54 and CD86 by flow cytometry) in order to select the best settings in terms of culture medium composition or treatment duration, for example. Then, the cellular interplay between the cell types of the co-culture is observed by analysis of the paracrine interaction using immunobassay. We used 7 reference chemicals to identify a gene signature from microarray analysis (whole human genome 60k). Finally, a differential extracellular release of cytokines (such as CCL3, CCL4, CCL5, CXCL1, CXCL8, CXCL10 and IL-18) is obtained and enables the distinction between sensitisers and non sensitisers. The VitroDerm / THP-1 co-culture is a promising model for skin sensitisation assessment and provides various advantages over ECVAM validated monoculture based methods. The presence of RHe within the co-culture allows closer replication of in vivo-like bioavailability, metabolism, keratinocyte inflammation as well as cross-talk between keratinocyte and dendritic cell surrogate, and thus contributes to modelling the natural process of sensitiser exposure to skin and activation of immune cells.

2432 Effects of Ammonia Vapor Exposure on Viability in a Full-Thickness Human Skin Tissue Model

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In this study, EpiDermFT, an in vitro 3D model of human skin, was used to evaluate the effects of ammonia vapor on skin to simulate in vivo exposure. The US Segment of the International Space Station (ISS) utilizes ammonia in the external loops of the active thermal control system. There is a low likelihood failure scenario that would allow ammonia to enter the habitable volume of the ISS via the interface heat exchangers. Protective masks have been developed to protect the eyes and respiratory tracts of crew in the event of a breach, but the dose/response for the effects of elevated ammonia vapor concentrations on intact human skin is largely unknown. Since EpiDermFT is cultured at the air-liquid interface and is amenable to topical exposures of test material, this 3D tissue model was an appropriate test system to simulate in vivo exposure to ammonia vapor. A custom exposure device was developed by Vitrocell and engineered to expose the apical surface of EpiDermFT tissues to either clean air (as a control) or ammonia gas at concentrations of 10, 100, 1000, 10,000, 30,000 or 50,000 ppm for 1, 5 or 20 minutes. Tissues were assessed immediately following exposure or 24 hours post-exposure for cell viability using the MTT assay and for morphological changes indicative of toxicity. Data indicate that concentrations between 10-1000 ppm ammonia for up to 20 min did not have an immediate or delayed (24 hr) effect on dermal tissue viability. Signs of toxicity, especially 24 hr post-exposure were noted at 10,000 ppm ammonia and increased in severity with both increasing exposure time and concentration. To our knowledge, this report represents the first evaluation of ammonia vapor on skin. Altogether, we demonstrate the utility of the EpiDermFT model as a relevant substitute of human skin for evaluation of cytotoxicity in response to environmental insults.

2433 Evaluation of the EpiSensA for Identifying Dermal Sensitization Potential of Complex Actives and Crop Protection Formulations

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The EpiSensA, developed by Kao Corporation, is emerging as one of the most promising in vitro assays for predicting dermal sensitization potential of chemicals. The assay predicts the sensitization potential based on the marker gene expression of differentiated three-dimensional reconstructed human epidermal keratinocytes, which have better metabolic capability and broader applicability domain compared to existing assays adopted to OECD TG. Previously, the EpiSensA demonstrated >90% accuracy for identifying known dermal sensitizers from 131 chemical dataset including lipophilic chemicals and pre/pro-hapten. In the present study, the performance of the assay was evaluated by testing a set of 9 materials (5 methacrylates, 3 silicone materials, and 1 crop protection formulation) that had prior in vivo studies (via LLNA and/or Guinea pig studies). The in silico models correctly predicted 6 of 7 materials, including a structural alert (false positive) for lauryl methacrylate. The DPRA and KeratinSens assays identified 6/9 (3 false negatives and 5/9 materials 3 false negatives and 1 false positive) correctly, respectively. The EpiSensA correctly predicted 8/9 materials with one silicone material as false negative. The false negative result for this silicone material, classified as weak sensitizer via LLNA, was consistent across the DPRA and the KeratinSens assays. Overall, consistent with prior evaluations, the EpiSensA demonstrated an accuracy level of 88.9% relative to available in vivo data. In addition, potency classification based on the concentration showing positive marker gene expression of EpiSensA was performed. The EpiSensA correctly predicted the potency for four out of five sensitizers classified as weak potency via LLNA (EC3 ≥ 10%). In summary, EpiSensA could identify dermal sensitization potential and potency of complex actives and crop protection formulations, and continues to show promise as an in vitro alternative for dermal sensitization.
The 3T3 Neutral Red Uptake (NRU) Phototoxicity assay is an established in vitro assay used to evaluate the potential phototoxicity of a test article. Since recommended by EMA and ICH guidance, the 3T3 NRU-PT assay has been used quite extensively in the pharmaceutical industry. The assay methods and prediction model are described in OECD Test Guideline (TG) 432 and the INVITTOX Protocol No. 78. In these documents, the following acceptable criteria for positive control chlorpromazine (CPZ) were defined: the $IC_{50}$ (+Irr) should be within the range of 0.1-2.0 $µg/mL$, the $IC_{50}$ (-Irr) should be within the range of 7.0-90.0 $µg/mL$, the photo irritation factor (PIF) should be $>6$. We used EBSS as the solvent to dissolve the CPZ in our facility and the historical data indicated that the $IC_{50}$ (+Irr) fall in the high end of recommended common range (actual range: 0.6675 to 2.861). We had observed that different solvents showed a significant impact on $IC_{50}$ value. In this abstract, the cytotoxicity and phototoxicity potential of CPZ dissolved in common solvents of DMSO and EBSS were evaluated for the impact. Two lots of CPZ were dissolved in DMSO and EBSS respectively. The solutions in DMSO were then diluted with EBSS at 1% (v/v) before treating cell cultures and the solutions in EBSS were directly used for cell treatment, the concentrations for both treatments are 0.0316, 0.1, 0.316, 1.0, 3.16, 10, 31.6 and 100 $µg/mL$. The results for two lots indicated that the cytotoxicity was obviously impacted by solvents but the phototoxicity potential of CPZ was not influenced. For lot 1 dissolved in EBSS, $IC_{50}$ (+Irr), $IC_{50}$ (-Irr) and PIF were 1.563 $µg/mL$, 45.91 $µg/mL$ and 29.38 respectively; while dissolved in DMSO, $IC_{50}$ (+Irr), $IC_{50}$ (-Irr) and PIF were 0.465 $µg/mL$, 12.54 $µg/mL$ and 26.988 respectively. For lot 2 dissolved in EBSS, $IC_{50}$ (+Irr), $IC_{50}$ (-Irr) and PIF were 1.169 $µg/mL$, 41.59 $µg/mL$ and 24.508 respectively; While dissolved in DMSO, $IC_{50}$ (+Irr), $IC_{50}$ (-Irr) and PIF were 0.4245 $µg/mL$, 9.807 $µg/mL$ and 23.142 respectively. Our data showed different solvents resulted in variabilities for $IC_{50}$ determination, we therefore suggest to remove $IC_{50}$ acceptable criteria or laboratories need to generate the $IC_{50}$ range based on specific solvent for this assay.

**2435 How to Assess a Phototoxicity Risk Related to Topical Exposure by Using the In Vitro SkinEthic RHE Model**

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The skin exposure to photoactive chemicals may produce abnormal skin reaction, an acute light-induced phototoxic response, which occurs when photoreactive chemicals are activated by solar lights and transformed into photoreactive molecules. This conversion of lasiocarpine is mainly catalyzed by CYP3A4, of which abundance and activity differs between individuals and ethnic groups. The aim of the present study was to predict the effect of inter-individual and inter-ethnic variation in liver toxicity of lasiocarpine predicted by integrating physiologically based kinetic (PBK) and Monte Carlo modeling.

**2436 A Study on the Correlation between In Vivo Tests and In Vitro Tests for Oral Mucosal Irritation Evaluation**

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Oral mucosal irritation is one significant element in the safety evaluation of oral care products. For an oral mucosal irritation test, the in vivo test method defined by the Cosmetic, Toiletry, and Fragrance Association (CTFA), is well known. However, we have examined the formation of ‘in vitro’ test method using a three-dimensional human buccal mucosal model as an alternative. We compared cytotoxicity of the in vitro test of oral care products and their ingredients and oral mucosal stimulation intensity of the in vivo test in order to confirm the correlation. For the in vitro test, a three-dimensional human buccal mucosal model EpiOral™ (manufactured by MatTek Corporation) was used. Among each toothpaste product and their ingredient of strong irritation, moderate irritation, and no irritation by in vivo test, we were selected as test substances. The test substances were applied to the pre-cultured EpiOral™ and rinsed out with PBS after exposure for a prescribed period of time. The cell viability was evaluated by the MITT assay at test concentrations and time points. We also evaluated the correlation between the irritation classification of test substances based on the cell viability and the in vivo data from modified CTFA method. In vitro data of oral care products and their ingredients tended to be consistent with the results of classification based on the oral mucosal stimulation intensity in the in vivo test. This suggests that it is possible to assess the in vivo test results from the in vitro test results, and it has been determined that the in vitro test is useful for oral mucosal irritation evaluation of oral care products.

**2437 Evaluation of Epiprostestinal™-Model for Absorption Studies In Vitro in an Automated Flow-Through Device**

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The Epiprostestinal™ model was developed by MatTek Corporation and is constructed as a 3D model, which reflects the anatomy of the gastrointestinal (GI) wall in the in vivo situation. Advantages of the model are its commercial availability, technical reproducibility and ready to use delivery. In the current project, we evaluated the Epiprostestinal™ model for the prediction of GI absorption in an automated technical flow-through system for 8 test labeled absorption tests (Antipyrine, Caffeine, Carbamazepine, Cimetidine, Ketoprofene, Metformin, Ranitidine and Warfarin) in respect of viability, barrier function, mass balance and absorption kinetics. Model validity was guaranteed by in-tact histology (in comparison to untreated models) and transepithelial electrical resistance (TEER) as well as absence of cytotoxicity (MTT measurements). Recovery of mass balance yielded 80 - 120 % of applied dose for 7 out of 8 test substances. Data were comparable between experiments (high and low dose). Papp values ranged from 2.7 (Metformin) to 60.4 (Antipyrine) $10^{-6}$ cm/sec and were comparable to literature data for 6 out of 7 compounds. Deviation between Epiprostintestinal™ model and Caco-2 was less than factor 3. Results were also comparable to ex vivo Ussing chamber experiments for Cimetidine, Ranitidine, Metformin and Antipyrine described in the literature (deviation below factor 3). The experimentally measured Papp values correlated with OSIAR calculated Papp values (deviation below factor 3) for 7 test-substances. Based on the findings described, Epiprostintestinal™ model is a promising tool for the determination of gastrointestinal absorption of test-substances in a fast, cost effective and technically easy to handle set up.

**2438 Inter-individual and Inter-ethnic Variation in Liver Toxicity of Lasiocarpine Predicted by Integrating Physiologically Based Kinetic (PBK) and Monte Carlo Modeling**

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Lasiocarpine is a pyrrolizidine alkaloid (PA) and able to cause liver toxicity following metabolic conversion to an unstable and reactive dehydro-PA metabolite. This conversion of lasiocarpine is mainly catalyzed by CYP3A4, of which abundance and activity differs between individuals and ethnic groups. The aim of the present study was to predict the effect of inter-individual and
2439 Mitochondrial-Destroying ToxCast Chemicals Inhibiting the Electron Transport Chain


Mitochondria generate the majority of cellular ATP through aerobic respiration and are central to cell proliferation, metabolic regulation, and apoptosis. Impaired mitochondrial function is linked to disease states such as Parkinson’s disease and metabolic syndrome; therefore, identifying mitochondrial-disrupting chemicals is important. We screened ToxCast Phase I/II chemicals using a modified Agilent Seahorse mitochondrial stress assay protocol with hepatocellular carcinoma HepG2 cells to identify compounds that altered basal, maximal, or inhibited oxygen consumption rates (OCR). Of 1,051 blinded test samples, 805 were inactive in a single-concentration format while remaining 246 samples were re-evaluated in a multiplex concentration format. These data were used to classify active chemicals as electron transport chain inhibitors (ETCi), uncouplers, or ATP synthase inhibitors. The largest classification group was ETCi with more than 120 chemicals. A subset of putative ETCi were mechanistically characterized using an electron flow assay to elucidate the inhibited ETC complex. Cells were permeabilized using a reagent that leave mitochondrial membranes intact and permitted the direct exposure to twenty suspected ETCi, each at three concentrations (31μM, 64μM, and 100μM). Mitochondria were fully uncoupled with carbonyl cyanide–4(trifluoromethoxy)phenylhydrazone (FCCP) to maximize OCR and initially provided glutamate and malate as complex I substrates. OCR was measured then challenged with three sequential injections: 1) suspected ETCi, 2) rotenone (complex I inhibitor)/succinate (complex II substrate), and 3) antimycin A (complex III inhibitor)/ascorbate and TMPD (complex IV substrates). The ETCi mechanism of each test chemical was elucidated using OCR recovery data. We determined that six chemicals specifically blocked complex I, three were specific to complex II/III, and five inhibited multiple ETC complexes. The ETCi mechanism of each test chemical was elucidated using OCR recovery data. We determined that six chemicals specifically blocked complex I, three were specific to complex II/III, and five inhibited multiple ETC complexes. This effort is the first of its kind to identify mechanisms of putative ETCi identified using a screening-based approach and will be expanded to include all identified ETCi with the ToxCast Phase I/II chemicals. This abstract does not necessarily reflect US EPA policy.

2440 The Power of Resolution: Contextual Understanding of Chemical-Biological Interactions

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Prediction of human response to chemical exposures is a major challenge in both pharmaceutical and environmental toxicology research. Transcriptomics has proven to be a powerful tool to explore chemical-biological interactions. However, limited throughput, high-costs and complexity of transcriptomic interpretations have yielded numerous studies lacking sufficient experimental context for predictive application. We utilized a novel high-throughput transcriptomics platform to explore a broad range of exposures to 24 reference compounds in both differentiated and undifferentiated human HepaRG cultures. Our goals were to 1) explore transcriptomic characteristics distinguishing liver injury compounds, 2) assess impacts of differentiation state on baseline and compound-induced responses (e.g., metabolically-activated), and 3) identify and resolve reference biological-response pathways and their quantitative translation to human exposures. Study data revealed the predictive utility of transcriptomic concentration-response modeling to quantitatively identify human liver injury compounds by their respective benchmark concentrations (BMCs), and model hepatic responses to classical reference compounds yielding plausibly-relevant estimations of human potency.

2441 Identification of Peroxisome Proliferators 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 28-day studies in rats. Over the last few years, 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 EU-funded Horizon 2020 project EU-ToxRisk, we have analysed the intracellular metabolome of HepG2 cells treated with two classes of peroxisome proliferators (phenoxy carboxylic acid herbicides: MCPP, MCPP, 2,4-D, 2,4-DP; and the phthalate DEHP and the corresponding mono ester MEHP). 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. In a principle component analysis, three variables were separated with regard to the control treatments along the first principal component. The metabolite profile shows an overall representation of lipid changes, indicating changes in lipid metabolism as can be seen for peroxisome proliferators in vivo. DEHP was not distinguishable from the control along the first principal component. This is probably due to the fact that p-hydroxy acids derived from the PPARs and need metabolic ester cleavage to the mono esters. Interestingly, DEHP and MEHP were separated from the controls along the second principle component indicating another biological activity in HepG2 cells. The data for the herbicides are well in line with the in vivo data as published in van Ravenswaay et al., 2016 and show that these in vitro data might be used to substantiate a read across based on in vitro methodologies.

2442 A Novel In Vitro All-Human Tri-culture Model That Maintains Structural Organization and Key Functions of Primary Hepatocytes over Several Weeks

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Predicting drug- and chemical-induced hepatotoxicity remains a great challenge. Animal-based testing during compound development too often fails to identify this risk, unable to mimic the role of non-parenchymal cells in response to compound perturbations and disease progression. Conventional in vitro model systems such as primary human hepatocytes lack or exhibit the loss of key functions as well as cellular architecture and integrity over time. We have developed an all-human tri-culture system, in both 24- and 96-well formats, comprised of primary human hepatocytes with endothelial and stromal cells derived from donated human tissues. Cell lines and densities and ratios were established based on the restoration and maintenance of key self-assembled cellular structures and functions over a 4 week period, measured by the localization of key cell-cell interaction and junctional polarity markers, albumin and urea synthesis, and CYP450 basal and induced activities. Experiments comparing mono- and tri-cultures of hepatocytes from multiple donors, healthy and diseased, demonstrated that basal albumin and urea synthesis rates and CYP450 basal and induced activities decreased markedly (>50% decrease of albumin and urea, undetectable levels of CYP450 basal expression due to cell loss) in monocultures. These cellular and biochemical parameters increased in the tri-cultures over the initial 4 to 7 days, reaching steady-state levels between 7 and 10 days on time and exceeding the healthy donor levels of albumin and urea expression maintained within a 20% range, and CYP450 fold change of >10. These promising results directly corresponded to hepatocellular remodelling facilitated by E-cad- and Cx32-mediated cell-cell interactions and cell junction and functional bile canalicular formation. Regardless of donor background, the presence of non-parenchymal cells sustained and enhanced hepatocyte integrity, polarity and performance over several weeks in culture.
Overall, this all-human tri-culture system represents an exciting and promising new analytical tool for studying the efficacy and liability of human compounds on hepatocellular function of both healthy and diseased human donors.

**2443 Studies on Physiological Equivalence of Human Hepatocytes and Liver Cells Harvested from Humanized-Liver Chimeric Mice**
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Human hepatocytes are an important tool for drug development and in vitro toxicity studies. However, due to the limited number of usable hepatocytes, it is difficult to perform reproducible studies using the same donor. To overcome this issue, we developed a TK-NOG mouse that makes it possible for human hepatocytes to proliferate within the host liver tissue. In this study, we isolated hepatocytes from TK-NOG chimeric mice with humanized liver (Hu-liver) and evaluated whether the hepatocytes could be used as alternative cells for primary human hepatocytes in in vitro toxicity studies. Purity and viability of the prepared hepatocytes (Hu-liver cells) were analyzed using flow cytometry. Up to 97% of the Hu-liver cells stained positive for human leukocyte antigen (HLA), with a mean viability exceeding 85% (n = 10). Monolayer cultured Hu-liver cells were bi-nucleated and displayed a cobblestone cell morphology. These are typical characteristics that are normally observed in human hepatocyte cultures. There was a good correlation between the mRNA expression levels of 16 P450 forms belonging to P450 subfamilies 1 to 4 in the Hu-liver cells and the expression levels in the human donor hepatocytes. The variations in individual P450 mRNA levels between the Hu-liver cells and donor human hepatocytes were within 5-fold for 13 P450 forms. Similar to the findings for the donor human hepatocytes, omeprazole/Rhodamined caused induction (>2.0-fold) of CYP1A2 and CYP3A4 mRNA in the Hu-liver cells. In contrast, there were only a few Hu-liver cell lots that showed induction (>2.0-fold) of CYP2B6, CYP2C8, CYP2C9, and CYP2C19 mRNA by the same inducers. Furthermore, we also performed a testosterone assay as a CYP3A probe to investigate the induction ability of the enzymatic activity in Hu-liver cells. After treatment with the CYP3A inducer, rifampicin, there was a significant increase in the production of 6β-hydroxytestosterone in the Hu-liver cells. These results demonstrated that Hu-liver cells have characteristics that are similar to those of human hepatocytes. These findings suggest that Hu-liver cells can potentially be used for in vitro toxicity studies.

**2444 Mode-of-Action Prediction of Liver Toxicants Using Metabolomics In Vitro**
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Analogous the in vivo metabolome database MetaMapTox (containing the profiles of >900 compounds), an in vitro liver metabolome platform using the liver carcinoma-derived HepG2 cell line has been established. This in vitro database contains the intracellular metabolite profiles of more than 80 toxicants with different modes of actions as well as negative substances. The modes of action for liver toxicity covered within the database range from peroxisome proliferation, liver enzyme induction, steatosis to general liver toxicity. To investigate the applicability of the platform for predicting the mode of action for liver toxicity of an unknown substance a double-blind study has been conducted with 4 treatments: Wy 14643, a peroxisome proliferator; Boscalid, an enzyme inducer; Carbon tetrachloride (CCL4), liver toxicity; Cidonion ethyl, a mixture of different modes of action and two structurally similar industrial chemicals without liver toxicity. All compounds were tested at two concentrations (minimal cytotoxicity and no cytotoxicity - spacing ca. 3 - 4-fold). Cells treated with the test substances for 48 h and the intracellular metabolome was determined using GC-MS and LC-MS/MS analysis. To evaluate the findings a profile comparison, which compares the metabolite profile of the respective treatment with the entire metabolite profiles of the reference compounds available in the database using Pearson correlation, was conducted. In addition, other statistical methods (calculation of euclidean and mahalanobis distances, hierarchical clustering and principle component analysis) were also used to determine the best prediction tool for this kind of data. The best overall prediction was obtained with the Pearson correlation analysis. Wy 14643 was correctly classified by all other tests as a peroxisome proliferator and Boscalid was correctly predicted by 3 out of 4 tests to be a liver enzyme inducer. CCL4 resulted in a mixed classification (Steatosis, Liver Enzyme Induction, general liver toxicity) as well as Cidonion-ethyl (Liver Enzyme Induction, Peroxisome Proliferation, general liver toxicity). The non-

**2445 Cytotoxicity and Oxidative Stress of Nivalenol and Sterigmatocstin on Human Hepatocarcinoma Cells**
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Sterigmatocystin (STE) is a carcinogenic mycotoxin, produced mainly by Aspergillus and Fusarium species, it is structurally similar to the aflatoxin B1. Nivalenol (NIV), produced by various Fusarium fungi, belongs to the trichothecenes group. Based on currently evidence, STE and NIV alone or combined can produce some toxic effects. The present study is conducted to investigate the cytotoxicity of STE and NIV as well as the possible mechanism under their toxicity on hepatocarcinoma HepG2 cells individually. Then the effects of STE and NIV were assessed in binary combination. The concentrations tested were from 0.156 to 25 µM for STE and from 0.156 to 5 µM for NIV. The highest cytotoxicity in HepG2 corresponded to NIV. The concentrations tested in combinations ranged from 0.78 to 12.5 µM (STE), and from 0.08 to 1.25 µM (NIV), with ratios of 10.1. The STE-NIV mixture exhibited additive effects. This study also explores oxidative cellular damage tested individually and combined by lipid peroxidation (LPO), reactive oxygen species (ROS) production and mitochondrial membrane permeability (MMP).

**2446 Development of a Multi-functional Fit-for-Purpose Rat Liver Co-Culture Assay for Haptotoxicity Testing**
ScitoVation, Durham, NC. Sponsor: P. McMullen.

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 with the CYP3A inducer, rifampicin, there was a significant increase in the production of 6β-hydroxytestosterone in the Hu-liver cells. These results demonstrated that Hu-liver cells have characteristics that are similar to those of human hepatocytes. These findings suggest that Hu-liver cells can potentially be used for in vitro toxicity studies.
Cardiotoxicity is a major safety liability for drugs, both in development and post-marketing, but has been largely neglected for environmental chemicals. Epidemiologic data suggest that about 35% of ischaemic heart disease burden is attributable to environmental risk, yet no in vivo or in vitro cardiotoxicity tests are required for non-pharmaceutical compounds. Moreover, the human population exhibits a large degree of variability in baseline cardiovascular risk, and data are lacking on the degree to which susceptibility to chemically-induced cardiotoxicity also varies. Human induced pluripotent stem cell (iPSC)-derived cardiomyocytes are an emerging in vitro model for cardiotoxicity screening of drugs. We have shown that inter-individual variability both in baseline cardiophysiologic and phenotypic characteristics as well as in drug-induced changes in beating parameters can be reproducibly modeled using a population of cardiomyocytes derived from different human donors. We therefore hypothesize that such an in vitro population-based model could be used to assess cardiotoxicity hazard, dose-response, and toxicodynamic variability for understanding the cardiotoxic liability of environmental chemicals. To test this hypothesis, we conducted concentration-response screening of 138 chemicals (drugs, pesticides, industrial chemicals, and food constituents) in iPSC-derived cardiomyocytes from 42 human individuals, comprising both sexes and differing ethnic backgrounds. We quantified kinetic calcium flux and high-content imaging data following chemical exposure, examined a range of cardiophysiologic and cytotoxicity phenotypes, and calculated point-of-departure (POD) values for each chemical and donor. We observed chemical-to-chemical variation in both potency and the degree of population variability. Treatment with known positive and negative drugs for cardiotoxicity exhibited in vitro phenotypes consistent with reported in vivo effects (or lack thereof). Additionally, consistent with previous studies using a single donor line, numerous non-pharmaceuticals showed cardiotoxicity with varying levels of potency. We observed inter-individual variability in responses across compounds, with ranges consistent with that observed in other population-based studies in vitro and in vivo. These results demonstrate the potential to use a population-based in vitro model to characterize not only cardiotoxicity hazard, but also inter-individual variability in susceptibility to such hazards.

**2448 Matured Human Cardiac Microtissues Provide Increased Predictive Value for Cardiac Toxicity Testing**

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In the ongoing efforts to reduce, refine and replace animal studies (3R), in vitro test systems are gaining increasing relevance. While classical 2D cell culture models are simple and easy to use, they fail to recapitulate more complex human tissue functions. The integration of human induced pluripotent stem cell derived cells (hiPSC) into microphysiologic system (MPS) helps to overcome these limitations as it enables better spatio-temporal control of culture conditions while allowing real time monitoring of toxicology studies. However, the immature electrophysiologic and structural properties of hiPSC-derived cardiomyocytes (CM) limits their utility for cardiotoxicity screens in the drug development pipeline. We recapitulated post-natal switching of the heart’s primary ATP source from sugar to fatty acids to enhance electrophysiologic maturation of hiPSC-CM. We hypothesized that this metabolic maturation will increase the predictive value of in vitro cardiac MPS. To this end, we have established a system mimicking in vivo heart muscle structure and function. By integrating force-sensing micropillars and electrode biosensors in our MPS system, we were able to derive the force of contraction and electrophysiology metrics in a non-invasive, paced, semi-real time, and cost-effective way. We identified fatty-acid based media that minimized the action potential duration while maximizing tissue contractile motion. MPS cultured in this optimized maturation media exhibit APD similar to that of adult human left ventricle. Interestingly, the same media did not alter the APD of hiPSC-CM monolayers. Matured cardiac MPS was superior to both hiPSC-CM monolayers and standard MPS in terms of its ability to predict safety margins for drugs that exhibit false positive toxicity (Verapamil), or false negative toxicity (Alfuzosin). With the addition of electrode and contraction force sensors, our matured cardiac MPS platform becomes much closer to being suitable for pharmaceutical pre-clinical toxicology trials in drug development processes and disrupts the current time consuming and costly drug approval steps, which will bring treatments to be commercialized and available to patients in a much faster and reliable way.

**2449 Evaluation of Corneal Damage Recovery Using 3-Dimensional Model**

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To advance the replacement of animal testing with alternative test methods, some OECD Test Guidelines (TGs) have been approved as alternative to in vivo Draize eye irritation test, where eye irritancy of substance is tested on animal’s eye. While it is not considered valid as a stand-alone replacement for the in vivo rabbit eye test, some test methods are recommended to identify chemical substances that do not require classification for eye irritation or serious eye damage as defined by the UN GHS (No Category) and may therefore be used as an initial step within the Bottom-Up testing strategy approach (OECD GD 263). However, a chemical substance that is not predicted as causing serious eye damage or as not classified for eye irritation-serious eye damage, that is, weak eye irritant such as UN GHS Category 2 with these test methods would require additional information to establish a definitive classification. In this kind of situation, we studied model and test method that can evaluate recoverability by using UN GHS category 2 substance. A 3-dimensional corneal reconstruction model was prepared with the immortalized corneal epithelial cell line (iHCE-NY1). The substances of differing eye irritation categories were applied to the model and recoverability by post culture was analyzed with cell viability and pathological finding using 30 substances. As results, all the substances by UN GHS category 1 were not recovered. On the other hand, frank tissue degeneration was observed by UN GHS category 2 substances almost 50%. The post culture of our corneal model may be the possibility to evaluate correctly the liquid substances by UN GHS category 1 and 2. Reference (OECD 2018). Guidelines for Testing of Chemicals. Organisation for Economic Cooperation and Development, Paris. Available at: http://www.oecd.org/chemistry/guidance-on-testing-chemicals.htm.


**2450 Molecular Footprints of Oxidative Stress in Corneal Injuries of Different Origin: Utilization of Human Organotypic 3D Corneal Tissue Model**

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Oxidative stress (OS) plays an important role in many corneal injuries (CI), including chemical burns, UVB irradiation, and exposure to vesicating agents. Current methods used to investigate mechanisms of OS utilize monolayer cell cultures or animals that result in poor data extrapolation and standardization, low throughput and high cost. Physiologically relevant, human-based in vitro models for ocular research are needed. This study evaluated the utility of an in vitro reconstructed 3D tissue model (Epicon) to study molecular mechanisms of OS corneal damage. The constructs are comprised of normal human corneal epithelial cells that are cultured at the air-liquid interface, develop a tight barrier (TEER 1000±250 Ω•cm²), express tight junctions, mucins, and key corneal detoxification enzymes similar to in vivo human cornea. OS was generated by exposure to 60/120 mJ of UVB, topical application of 20/50 mM of hydrogen peroxide (H2O2) for 2h or 1.5 mg/ml of nitrogen mustard (NM, warfare agent) for 10/30 min. Barrier function, tissue viability, reactive oxygen species (ROS), lipid oxidation, cytokine release, histology, and gene expression were evaluated after 2/24h post-exposure. HP reduced tissue barrier to 50% and viability (MTT assay) to 70% after 2h incubation compared to negative control (NC). NM reduced tissue barrier to 76%, however tissue viability was not affected after 2h. 20 mM HP- and 10 min NM-treated tissues recovered after 24h, while 50 mM HP- and 30 min NM-treated tissues degraded as confirmed by TEER, MTT assay, LDH release, and histological analysis. UVB irradiation didn’t have an effect on tissue barrier, viability, and LDH release, but intracellular ROS increased by 17x when compared to the NC. The highest lipid oxidation (20 X1 increase) and IL-8 release (18.6X increase) relative to NC were observed for 10min/NM-treated tissues at 24h post-treatment. Utilizing a PCR gene array we compared effects of different CI corneal injuries on the expression of 84 OS responsive genes, and found a specific molecular footprint for each mechanism of corneal injury. In summary, the in vitro reconstructed human corneal tissue model structurally and functionally reproduced key features of in vivo corneal tissue. Unique toxicity and molecular responses were
revealed for various types of ocular damage. This model is anticipated to be a useful tool to study molecular mechanisms of corneal damage and to evaluate new corneal drug formulations.

2451 The EYEIRR-IS Assay: Development of an In Vitro Method Using SkinEthic HCE Model for Liquid Chemical Eye Irritation Sub-categorization

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Over the last 20 years, many efforts have been made to find reliable and relevant alternative methods to evaluate eye irritation hazard. Although several in vitro and ex vivo test methods have been regulatory accepted by the OECD for the identification of either UN GHS Category 1 (e.g. BCOP) or No Category chemicals (e.g. SkinEthicHCE EIT), none of them could distinguish chemicals that cause eye severe damage (Cat 1) from those causing eye irritation (Cat 2). Using a toxicogenomic approach on the human 3D corneal epithelial model SkinEthicHCE, we developed a new assay: EYEIRR-IS, to subcategorize liquid chemical irritants into Cat 1 or Cat 2 subclasses. An optimized protocol based on a 10 minute treatment followed by 6 h post-treatment incubation on 2 concentrations of liquid chemicals (100% and 30%) was assessed on a balanced set of 18 substances (4 Cat 1, 5 Cat 2, 9 No Cat referenced in the OECD reference chemicals list). A prediction model based on the analysis of 10 specific genes overexpression level to calculate a Liquid Irritation Index was used to sub categorize the liquid chemicals. The preliminary data showed promising results, with the correct identification of all Cat 1 and Cat 2 chemicals. The majority of the known in vivo non classified chemicals (7 out of 9) were well classified in vitro. These results suggest that the EYEIRR-IS test method may be a promising in vitro tool for the assessment on liquid chemicals of the irritation potential in all of the GHS classes. Further experiments are ongoing to further assess the predictivity of EYEIRR-IS on a larger set of chemicals.

2452 Identifying Eye Irritants (GHS Category 2) Using Validated, Nonanimal Tests


OECD Test Guideline 405 prescribes a weight-of-evidence (WoE) analysis and sequential testing strategy for the classification of acute eye hazards, including: “Perform validated and accepted in vitro or ex vivo ocular test(s).” Four tests qualify: three designed to identify severe eye irritation/corrosion (GHS Category 1) and one to identify non-irritants (GHS No Category). GHS Category 2 (eye irritant) classification is impossible using any single test. The EpiOcular Eye Irritation Test; EIT (OECD 492) classifies a substance to be No Category, or contrarily causes (uncategorizable) eye effects. Conversely, the Bovine Corneal Opacity and Permeability (BCOP) Test (OECD 437) is used for ruling in or ruling out Category 1 effects. By using a dual-assay/approach system - the combination of the EIT and BCOP test - we have determined, with a high degree of accuracy, GHS Acute Eye Hazard Category 2 chemicals that cause reversible eye irritation. When a BCOP test rules out GHS Category 1, and the EIT rules out GHS No Category, analysis of these results indicates the only other possible designation - Category 2. Per GHS, Category 2 classification defaults to Category 2A, because differentiation between Category 2A and 2B cannot be made. After testing 42 chemicals, we correctly identified 93% of the Category 2A/B chemicals as Category 2. The potential of the BCOPETI dual-assay system, coupled with WoE evaluation, to correctly classify substances into GHS Category 1, Category 2A, and No Category is encouraging. We predict using this testing strategy would greatly reduce reliance on Draize Rabbit Eye Tests.

2453 Prevalidation of the OptiSafe Ocular Irritation Assay for the Detection of Ocular Corrosives

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OptiSafeTM is a novel, shelf stable, test tube-based assay that can discriminate ocular irritants/corrosives from nonirritants and does not use animal tissues or cells. It can determine whether an unknown substance is an ocular nonirritant by measuring and comparing chemical models for three variables: damage to the corneal stroma, damage to phospholipid bilayers, and the potential to induce pH extremes in a system. A substance can be evaluated in less than 2 hours, and results can be obtained in fewer than 24 hours. Previous studies indicated that predictions of EPA Category IV or GHS category NC (not classified) by the OptiSafe ocular irritation assay had high sensitivity, suggesting that it may represent a new tool for the in vitro assessment of the ocular toxicity potential of chemicals in a tiered-testing strategy. While a limited number of ocular corrosives have been tested, the initial analyses of retrospective and prospective OptiSafe data suggest that the test method is also sensitive for the detection of ocular corrosives. OptiSafe correctly predicted, with 100% specificity, the sensitivity was 87.5% and the specificity was 70%. The EPI Category I prediction yielded a sensitivity of 85.7% and a specificity of 67.6%. OptiSafe had a higher sensitivity for the detection of ocular corrosives and similar or lower specificity compared to other in vitro test methods: Bovine Cornea Opacity/Permeability Assay; EpiOcular; Short-Time Exposure. To confirm the results of these initial studies, additional ocular corrosives will be assessed to provide a more complete description of the sensitivity and accuracy for the detection of ocular corrosives. If the validation results are confirmed, then OptiSafe may represent a new approach that moves closer to the in vivo category test paradigm. NC/IV classification results can be accepted with a higher level of confidence, and noncorrosive results can be accepted with an above-average level of confidence. While positives may require confirmation by other methods, nonirritant and noncorrosive results may be acceptable. This approach may find applications in scenarios where there is a time-sensitive need to determine whether a substance is safe for eye area exposure. This project was funded in whole or in part with federal funds from the NIH under Contract No. HHSN27201500010C.

2454 Updated Survey of CAMVA and BCOP Assays Used to Predict Ocular Irritancy of Personal Care Products—7 Years Later


Kao USA Inc has used the combination of bovine corneal opacity and permeability (BCOP) and chorioallantoic membrane vascular assay (CAMVA) in vitro to assess ocular irritation since 1996. In 2011, Kao USA created a decision tree to outline when to conduct ocular testing of new cosmetic formulations. Here, data from 150 BCOP and CAMVA studies, conducted since 2011, were surveyed to 1) confirm the decision tree continues to accurately predict the irritant classifications of certain product categories and 2) determine if any decision tree updates are required. The new data predict hair shampoos, skin cleansers, and ethanol-based hair styling sprays are ocular irritants, confirming the original predictions. Conversely, the new data predict artificial sweeteners are non-irritants, also consistent with the original predictions. The accuracy rate of decision tree predictions for these product categories is 89.7% (78/87 products). The false negative rate is 8% (7/87), but is explained by deeper examination of individual BCOP scores and CAMVA IRCt values. For example, some tested products that have irritant BCOP scores near the non-irritant threshold and have non-irritant CAMVA scores are not considered irritants because the BCOP assay has a known limitation in discerning between mild irritants and non-irritants. Hair conditioners were not included in the original decision tree due to wide variability in the original test results surveyed. This work includes a review of 52 conditioners with new data that 1) new conditioners are predicted to be non-irritants and supports a potential update to the decision tree. Ocular testing decision tree predictions are one piece of information used in a conservative weight-of-evidence framework to classify cosmetic formulations. Ongoing re-reviews of the resulting predictions are planned to ensure continued accuracy of the decision tree predictions, to strengthen existing and future conclusions, and to ultimately support a robust safety assessment of cosmetic formulations.

2455 In Vitro Metabolome Analysis Can Predict Nephrotoxicity and Its Mode-of-Action

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Nephrotoxicity and its mode of action (MoA) is a highly relevant toxicological endpoint for chemicals. Therefore, reliable and robust in vitro methods are the preferred methods for screening and thorough mechanistic understanding in the regulatory context. A tubular fibroblast rat cell line (NRK-52e) is cultivated on Lumox dishes (Sarstedt), treated for 48h and sensitively harvested. LC-MS/MS and GC-MS/MS technology was applied to quantify 353 endogenous metabolites. 9 different nephrotoxic compounds are the preferred methods for screening and thorough mechanistic understanding. A new nephrotoxic test system was developed to further assess the predictivity of EYEIRR-IS on a larger set of chemicals.
Evaluation of a Method for Assessing Respiratory Toxicity in vitro

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Ortho-phenylaldehyde (OPA) is a liquid chemical stunt used for the high-level disinfection of heat-sensitive medical devices, such as endoscopes and microsurgical instruments. Although viewed as a safer alternative to glutaraldehyde, a known skin and respiratory sensitizer, an OPA exposure limit to control associated health risks from inhalation exposure has not been established. To simulate human OPA inhalation exposure, we treated a human airway epithelial cell line (A549) to an in vitro aerosol. A single exposure to OPA aerosols at the air interface with a single dose of OPA aerosols prepared from solutions containing 0 (control), 0.2, 0.5 and 1.0 mg/ml OPA; tissue responses were evaluated at 20 min, 24 h and up to 5 days later. Treatments with 0.2 and 0.5 mg/ml OPA were non-cytotoxic and significantly increased trans-epithelial electrical resistance (TEER). 1.0 mg/ml OPA, however, was cytotoxic based on the lactate dehydrogenase (LDH) release assay and TEER measurements. Except for apical LDH release, membrane leakage from the basolateral side and TEER returned to baseline following a 5-day recovery. Single OPA exposures decreased the levels of reduced (GSH) and oxidized glutathione (GSSG), lowered GSH/GSSG ratios and upregulated the expression of heme oxygenase-1, indicating induction of oxidative stress. Dose-dependent functional disruptions, such as inhibition of cilia beating frequency (CBF), decreases in MUC5AC secretion and aberrant cytokine secretions were observed 24 h following single exposures. Measurements made up to 5 days following the exposures indicated that alterations in CBF, MUC5AC secretion and cytokine secretion only partially recovered from the treatments, while MUC5AC secretion and induction of aldo-keto reductase 1B10 persisted over the same timeframe. No morphological changes were observed following a single exposure. Taken together, these findings suggest that OPA exposure induces respiratory epithelial irritation. Our findings will help support setting a limit to inhalation exposure for this chemical as a high-level disinfectant.
2460 Validation of an In Vitro Alternative Method for Acute Respiratory Toxicity Testing

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Determination of acute respiratory toxicity potential is important for establishing safe use of chemicals and consumer products. Inhalation toxicity testing and classification procedures currently accepted by worldwide government regulatory agencies require the use of tests based on lethal effects in animals. The current work provides an update on the development and validation of an alternative non-animal method for determining acute respiratory toxicity using the EpiAirway™ in vitro human airway model. The in vitro test method exposed EpiAirway tissues to four concentrations of test chemicals for three hours via apical application using either aqueous or corn oil vehicles. After the initial three hour exposure, the test chemicals were rinsed off and the tissues were incubated for an additional 21 hours. An IC75 concentration (concentration required to reduce the endpoint value to 75% of vehicle exposed controls) was determined from the dose-response data using barrier function (determined by measuring transepithelial electrical resistance (TEER)) and tissue viability (MTT assay) as endpoints. Based on the determined IC75 value, EpiAirway tissues were exposed to six serial dilutions of the test chemicals, using the IC75 as the highest dose. Tissues were apically exposed for three hours, followed by rinsing, every Monday, Wednesday and Friday, with TEER measured prior to each dose application. Experiments were continued for at least 30 days to determine no-observed-adverse-effect level (NOAEL) doses. Experiments conducted with formaldehyde, ethanol and dimethylacetamide found NOAEL values of 0.0001, 0.5 and >0.5 mg/ml, respectively. Expansion of the data set to include chemicals of different classes, chemical structures and physical properties is ongoing. These results indicate that in vitro airway tissue models using TEER as a convenient non-destructive endpoint are a promising alternative to animal tests for assessment of chronic respiratory toxicity.

2462 Development of a Chronic Respiratory Toxicity Assay Using an In Vitro Human Airway Model

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Knowledge of chronic respiratory toxicity potential is important for establishing safe use of chemicals and consumer products. The current work describes efforts to develop an alternative, non-animal method for determining chronic respiratory toxicity using the EpiAirway™ in vitro human airway model. Initial acute toxicity experiments were conducted by exposing EpiAirway tissues to four concentrations of test chemicals via apical application using either aqueous or corn oil vehicles for three hours. After exposure, the test chemicals were rinsed off and the tissues were incubated for an additional 21 hours. An IC75 concentration (concentration required to reduce the endpoint value to 75% of vehicle exposed controls) was determined from the dose-response data using barrier function (determined by measuring transepithelial electrical resistance (TEER)) and tissue viability (MTT assay) as endpoints. Based on the determined IC75 value, EpiAirway tissues were exposed to six serial dilutions of the test chemicals, using the IC75 as the highest dose. Tissues were apically exposed for three hours, followed by rinsing, every Monday, Wednesday and Friday, with TEER measured prior to each dose application. Experiments were continued for at least 30 days to determine no-observed-adverse-effect level (NOAEL) doses. Experiments conducted with formaldehyde, ethanol and dimethylacetamide found NOAEL values of 0.0001, 0.5 and >0.5 mg/ml, respectively. Expansion of the data set to include chemicals of different classes, chemical structures and physical properties is ongoing. These results indicate that in vitro airway tissue models using TEER as a convenient non-destructive endpoint are a promising alternative to animal tests for assessment of chronic respiratory toxicity.
2464 Authentic Lung and Gingival Fibroblasts Cell Models for In Vitro Toxicity Testing

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There is increasing demand for in vitro models for toxicity testing to replace animal models. Drivers for this change include decreased overall cost for cell-based models and the ability to do more high throughput screening. Development of cell based in vitro models for toxicity testing is a challenging task. Primary cells can best represent the in vivo situation, however, donor variability and replicative senescence restrict the potential usefulness of this cell model in the study of toxicity. Conversely, continuous cell lines may lose their relevant physiologies and thus their usefulness. Human telomerase reverse transcriptase (hTERT)-immortalized primary cells provide a better solution: cells can be continuously subcultured while retaining the physiological characteristics of the parental primary cell. In this study, we established two clonal hTERT immortalized cell lines by expressing hTERT in lung fibroblasts and gingival fibroblasts isolated from normal donors. Both lines have been cultured continuously for more than 35 population doublings without any signs of replicative senescence. Further, both hTERT-immortalized fibroblasts retained normal diploid karyotype over extended culture period, demonstrated no indication of malignant transformation, and maintained typical fibroblast characteristics (e.g., positive TE staining, a fibroblast marker; and negative K14 staining, an epithelial cell marker). Both cell lines responded to TGF-beta treatment with elevated smooth muscle actin expression as did the parental cells. Notably, both cell lines are sensitive to toxicological agent (Chlorhexidine) treatment, and showed similar patterns as their primary counterparts in a dose-dependent manner. The results show that the hTERT immortalized lung fibroblasts and gingival fibroblasts cell lines retain the important physiological characteristic of the primary cells from which they were derived and provide very useful in vitro cell models for toxicity screening.

2465 Development of a Fit-for-Purpose In Vitro Model of Lung Toxicity


A shift in toxicity testing from in vivo animal models to in vitro human cell-based models is a major focus of current research, due to the high cost, low throughput, ethical concerns, and questionable human relevance of in vivo testing. While many high throughput in vitro assays for biological perturbations do exist (as illustrated by the ToxCast effort), it is clear that more physiologically relevant cellular models are required to reproduce adverse outcomes. The lung epithelium is a frequent target of chemicals under regulation for toxic effects, yet efforts to develop in vitro lung models fit for the purpose of toxicity testing have been relatively few. To reproduce such adverse effects as proliferation and fibrosis, it will be necessary to utilize cellular models of the lung that incorporate multiple differentiated cell types growing at the air-liquid interface (ALI). Furthermore, to ensure interpretable dosimetry, exposure of cells to test compounds must be via the air. We are exploring testing approaches that meet these criteria. As a proof-of-principle experiment, we have treated primary human bronchial-tracheal epithelial cell lines (HBTECs), grown at ALI, with chloroform vapor using the Vitrocell inhalation exposure system. Chloroform is metabolized by CYP2E1 to form reactive intermediates, including phosgene, which react with glutathione GSH). Metabolically competent cells are expected to be indeplete GSH through conjugation as well as oxidation to GSSG; therefore, we chose to monitor oxidized and total GSH after exposure. To identify exposures likely to span the point-of-departure for this endpoint, we first performed a range-finding experiment with A549 human alveolar carcinoma cells treated with chloroform in solution for 24 hours. 1 μM had little effect on total and oxidized GSH levels compared to vehicle, whereas 25 μM greatly increased oxidation. Chloroform concentrations bracketing this POD (2.5, 10, and 40 μM) were selected and converted to equivalent 1 h vapor-based exposures, (69, 267, and 1069 ppm, respectively), by matching the nominal AUCs of the media and vapor-based exposure using a blood/aire partition coefficient. HBTECs grown at ALI were then treated with increasing concentrations of chloroform vapor for 1 or 3 hours. At both timepoints, GSH levels were significantly reduced, and GSSG/GSH ratios were increased, at 1000 ppm chloroform compared to clean air treatment. This result demonstrates the promise of this system for in vitro inhalation safety testing.

2466 Interference of Barcoded DNA Measurements When Determining High-Throughput CYP-Mediated Cytotoxicity

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Many cell lines used in current high-throughput chemical testing lack chemically metabolized, and therefore may misclassify the hazard potential of metabolites relevant to human exposures. To help address this challenge, we engineered HEK293T cells to overexpress cytochrome P450 monoxygenase (CYP) recombinant transgenes commonly involved in chemical metabolism within the human liver (CYP1A1, CYP1A2, CYP2E1, and CYP3A4). Each clone was treated with unique untranscribed DNA barcode, which can distinguish each clone in a mixed clonal culture by PCR and serve as a quantitative measure of cell number. This method allows adaptation for high-throughput assessments of chemical induced cytotoxicity thereby specific CYP-mediated bioactivation. As a proof of concept, we analyzed clonal toxicity to a potent hepatotoxicant, aflatoxin B1 (AFB1). Traditional cytotoxicity assays confirmed AFB1 bioactivation and greater toxicity in CYP3A4- and 1A1-expressing clones (62% and 31% cell loss compared to controls at 80 μM AFB1, respectively). Surprisingly, when clone-associated DNA barcodes were measured by droplet digital PCR (ddPCR), we observed significant increases in CYP3A4 and 1A1 barcode counts after AFB1 exposure (by 4.5-fold and 2.5-fold compared to controls, respectively) incorrectly suggesting detoxification rather than bioactivation. We speculate the intact, normal DNA from fresh cell lysate restricts probe access more than the fragmented DNA present in apoptotic cells leading to higher barcode readings. To address this issue, optimized homogenization of sample and direct ddPCR measurement in the cell lysate reduced variance; however, increased barcode counts in clones sensitive to AFB1 were still observed. Moreover, exposure to a known apoptosis-inducer in these cells, staurosporine, resulted in the same phenomenon. Mechanism of cell death is currently being assessed as the primary factor in the observed aberrant barcode measurements. This abstract does not necessarily reflect US EPA policy.

2467 Exploring the Feasibility of A549 Cells for Use in a Vapor Exposure Test System for Acute Toxicity Evaluation


The 3M Strategic Toxicology Laboratory (STL) is an internal corporate resource providing support to 3M businesses that emphasizes the use of in vitro methodology. The purpose of the current study is to validate an in vitro acute vapor inhalation model using the VitrocellTM vapor exposure system and A549 adenocarcinomic human alveolar basal epithelial cells. Multiple variables were examined including seeding density, airflow rate, growth duration in submersed culture, and subsequent time at the air-liquid interface (ALI) prior to exposure. Cell viability was assessed using the MTT assay. Results indicated that a seeding density of 500,000 cells/well grown for 7 days with the apical surface submerged provided the optimal optical density (OD) readings between 2.0-2.5, which provided sufficient sensitivity to evaluate viability. To evaluate the incubation time at the ALI, A549 inserts that were freshly brought to the ALI or were cultured at the ALI for 14 days prior to use, were exposed to increasing concentrations (0-12,000 ppm) of trans-dichloroethylene (t-DCE) for 3 hours. t-DCE vapor was chosen because of its intrinsic low toxicity in vivo, and concentrations were measured with a micro-GC sampling system continuously throughout the exposure. A549 cells moved to the ALI immediately before t-DCE exposure demonstrated 30-40% viability after 4,000, 8,000, and 12,000 ppm exposures, compared to incubator controls. Further, visual observations revealed that the center of each insert, directly under the air inlet in the Vitrocell, appeared to be non-viable, including the clean air controls. In contrast, cells that adhered to the ALI and allowed to continue growth in the incubator for 3 or 7 days then exposed to clean air at a flow rate of 1.2, 3 or 5 ml/min, or 14 days before t-DCE exposure were 80-90% viable compared to incubator controls, and non-viable centers were not observed. In summary, a seeding density of 500,000 A549 cells/well was optimal for this model. Conditioning the ALI by lifting them to the ALI for an additional 7-14 days prior to use made them more tolerant to the exposure conditions within the Vitrocell. While these data provide a starting point for the use of A549 cells to evaluate acute toxicity screening within the Vitrocell vapor exposure system, further studies are needed using a variety of known in vivo toxicants to better characterize the utility of this in vitro test system in screening for vapor toxicants.
Beauvericin and enniatin B are two emergent mycotoxins from Fusarium fungi which are frequently detected concomitantly in cereals and cereal-based products. They have been studied individually in vitro and in vivo, showing contradictory toxicological results. While in vitro they have shown ionophoric activity and subsequent mitochondrial toxic properties among others, in vivo only slight adverse effects, as loss of weight, have been found. At transcriptome level in Jurkat lymphoblastoid T-cell line, it revealed a similar down-regulation pattern affecting most of the genes involved in the electron transport chain pathway. In order to delve into the adverse outcome pathway that leads to homeostasis triggered by these mycotoxins, it was proposed to investigate the changes in mitochondrial protein expression in Jurkat cells. The chosen combination of beauvericin and enniatin B was 1:1 at three different doses: 0.01 - 0.1 - 1.5 µM. Control cells were exposed to 0.5% DMSO. Cells were treated during 24 h and then mitochondria were extracted. Three biological replicates and technical duplicates from each treatment were injected in a Nano-LC Q-TOF UHPLCMS and raw data were processed using Swissprote database limited to human entries. After comparing the control and the three doses results, from the 1821 proteins identified and quantified, 340 proteins were selected using the parameters: max fold change ≥ 1.5 and Anova p-value ≤ 0.05. These selected proteins were analyzed by different bioinformatics tools for proteomics data interpretation. The most overrepresented pathways in Jurkat lymphoblastoid T-cell line was the citric acid cycle and respiratory electron transport, mitochondrial protein import and respiratory electron transport, ATP synthesis by chemiosmotic coupling, and heat production by uncoupling proteins. PANTHER version 13.1 indicated ATP synthesis as the most overrepresented pathway and mitochondrial ATP synthesis coupled proton transport as the gene ontology biological process. These results suggest that mitochondria are the main target for beauvericin and enniatin B mixture toxicity. Acknowledgements: This work was supported by the Spanish Ministry of Science, Innovation and Universities (AGL 2016-77610R).

Pyrogens are substances that can produce fever when present as contaminants in a drug or medical device; most pyrogens are biological substances derived from bacteria, fungi, and viruses. Material-mediated pyrogens (MMPs), while less common, may also be present. It’s important that medical device products for implantation or other systemic exposure meet pyrogen limit specifications before they are marketed, and animal-based pyrogen tests (i.e., LAL and rabbit pyrogen tests) are often conducted to investigate the presence of pyrogens. Non-animal pyrogen activation tests (MAT) are widely available but infrequently used. To review the MAT and discuss ongoing challenges to its widespread implementation, NICEATM and the PETA International Science Consortium (PISC) co-organized a September 2018 workshop. Workshop participants explored how the US FDA’s Medical Device Development Tools (MDDT) Program could be used to qualify the use of MAT as a standalone pyrogen test for specific medical device contexts of use. Attendees discussed practical aspects of pyrogen testing and the evidence needed to support qualification of MAT as a replacement for animal-based pyrogen tests. Scientists from the US FDA Center for Devices and Radiological Health (CDER), the US EPA, and consultants for use of the MAT in assessment of medical device biocompatibility and sterility and the role of the MDDT Program for qualification of MAT. There was general agreement that the MAT likely could be qualified as acceptable for batch-release testing for microbial-based pyrogens. However, additional studies were recommended to demonstrate its ability to detect known MMPs. This testing would determine whether the MAT can be used for both biocompatibility and sterility or if other information on MMPs would be needed to address biocompatibility. Participants also discussed information gaps on MMPs, potential test controls, and other challenges and opportunities for implementing the use of MAT as a comprehensive pyrogen test. Funded in whole or in part with federal funds from the NIEHS, NIH under Contract No. HHSN27220150010C.

Flavorings in e-cigarette liquids are a primary driver of e-cigarette use among teens. While recent studies have investigated pulmonary toxicities associated with exposure to flavorings in e-cigarettes, little research has been conducted on the impact of flavorings on the pharmacokinetics of nicotine, the primary addictive compound of e-cigarettes. Utilizing recombinant CYP2A6, the major enzyme responsible for the metabolism of nicotine, we screened 3 e-liquids, Strawberry Poptart (SP), Apple Watermelon (AW), and Flamethrower (FT) at concentrations ranging from 0.0025% to 0.25% (v/v), for CYP2A6 activity inhibition. CYP2A6 activity was determined by measuring the formation of the fluorescent metabolite of 3-cyano-7-hydroxycoumarin, a substrate of CYP2A6. Incubation with FT and SP strongly inhibited CYP2A6 as low as at 0.0025%, while AW exhibited no CYP2A6 inhibition. GC-MS analysis was conducted on FT and SP to identify compounds responsible for CYP2A6 inhibition. Trans-cinnamaldehyde, a potential inhibitor of CYP2A6, was identified in FT, while benzaldehyde was identified in SP. We subsequently screened both aldehydes using recombinant CYP2A6 at concentrations ranging from 0.25μM to 250μM. Trans-cinnamaldehyde and benzaldehyde exhibited near maximal inhibition of CYP2A6 at 2.5μM and 25μM, respectively. To confirm our findings in a cell-based system, we developed a novel in vitro cell culture apparatus capable of screening volatile compounds when heated in an incubator-something not feasible in traditional cell culture systems. In the novel culture system, BEAC-2B cells transduced to overexpress CYP2A6 were cultured and subsequently exposed to e-liquids and flavoring compounds in the presence of nicotine. We quantified nicotine levels using LC-MS. Exposure to SP, FT, and both flavoring aldehydes significantly reduced the metabolism of nicotine by CYP2A6 at concentrations consistent with what was observed using recombinant CYP2A6. In summary, these data indicate that some flavoring compounds in e-cigarettes have the capacity to inhibit CYP2A6 activity, the enzyme responsible for catalyzing nicotine. This may increase serum concentrations of nicotine and have implications for the risks associated with e-cigarette use. This abstract may not reflect official US EPA policy.

Unhealthy diet and lack of exercise are major contributors to weight gain and obesity; however, recent studies have suggested that environmental exposures, so-called “obesogens”, may also play a role. It has been proposed that exposure to certain agents early in adipose tissue development may increase the susceptibility to metabolic syndrome later in life by disrupting hormone homeostasis and changes in adipocyte differentiation, thereby increasing the fat storage capacity and/or the number of fat cells. In vitro systems to identify potential obesogenic chemicals are a cost-effective strategy to identify potential hazards. Here, we tested a recently developed white adipose tissue (WAT)-on-a-chip system with a footprint of less than 1 mm² consisting of a separate media channel and WAT chamber connected via a small microporous membrane. WAT MPS technology was transferred from the University of California at Berkeley to Texas A&M University and then utilized as MPS platform to study the effects of chemicals on adipogenesis. Two cell lines, 3T3-L1 mouse fibroblast and human subcutaneous preadipocytes were studied in both traditional 2D culture and in 3D WAT MPS. Dexamethasone, triamcinolone, mifepristone, diethyl sulfate, and GW9662 were tested in concentration-response fashion based on their purported effects on glucocorticoid receptor (GR) and PPAR-regulated pathways. Maturation of adipocytes was quantified using markers including lipid accumulation, adiponectin secretion, expression of key genes, free fatty acid release, and glucose uptake. We demonstrated successful technology transfer of a complex MPS between laboratories and established a functional and viable adipose tissue in the WAT platform for over two weeks in culture. We found that dexamethasone and triamcinolone had effects on adipogenesis in both traditional 2D culture and 3D MPS platform. This research was funded by grants U24 TR001950 and UH3 TR000487.
2472 New Screening Approach of Chemicals—Investigation of Retinoic Acid Receptor Alpha (RAR Alpha) Interaction by Combining Two Different Techniques


Reproductive toxicity is one of the most sensitive endpoints within regulatory toxicology of chemicals. Identification of reproductive toxicity and the mode of action is important. Changes of the retinoic acid receptor alpha (RARα) hemostasis can lead to adverse effects including teratogenity. The RARα is a nuclear receptor present in the cellular nucleus and bound to the retinoic acid responsive elements in front of target genes. A key step in the ligand induced transcription of those controlled genes is the recruitment of cofactors by RARα. A cell-based system in transfected HEK293 cells, expressing RARα (BPS Bioscience) and a micro array system called MARCoNi (PamStation®) were compared and the strength and limitations to identify RARα-interaction were determined. Six known agonists (e.g. A2M801), four antagonists (e.g. BMS195614) and four non-responders (e.g. Ketaconazole) were tested with both methods. The dose response of peptides/cofactors, known as relevant for the RARα interaction, corresponded nicely to the response of the HEK293 cell line. Exemplarily: after ATRA treatment 16 selected cofactors/45 peptide motifs revealed ECO50 values between 6.6⋅10⁻⁸ M and 1.0⋅10⁻⁸ M and was comparable to ECO50 value of 1.9⋅10⁻⁷ M for ATRA in HEK293 cells. While the HEK293 cell line is suitable to determine the agonistic and antagonistic property of a chemical in an easy and economical way, the experiments in the MARCoNi-system revealed the molecular initiating event by influencing corepressor or coactivator expression. Since the agonistic effects are identified properly with both methods, the MARCoNi-system allows a better understanding of mechanistical processes for diverse antagonists. The combination of both methods increases the value of each individual method by compensation of the limitations of each other. Based on this a testing strategy was implemented at BASF to investigate the RARα interaction including mechanistical understanding of chemicals in the screening phase of new chemicals.

2473 Alginate Immobilization of Metabolic Enzymes (AIME) Coupled to an Estragon Receptor Transactivation Assay Detects Bioactivated and Bioinactivated Estragon Receptor Agonists

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The Alginate Immobilization of Metabolic Enzymes (AIME) is a high-throughput-screening (HTS)-compatible platform that retrofits existing in vitro assays with metabolic competence by attaching alginic-hepatic S9 microspheres to solid supports extending from custom microplate lids. We have previously demonstrated that the AIME platform can be coupled with the VM7Luc4E2 estrogen receptor (ER) transactivation assay using methoxychlor as a reference chemical for bioactivation to a more potent ER agonist. In this study, we selected 34 compounds reported to have more ER activity after bioactivation. The changes were already seen only at 12h of exposure and were more expressed for the majority of the hits was below the compound-dependent cytotoxicity cutoff, indicating relevant bioactivity. These data demonstrate the metabolism-coupled AIME-VM7Luc4E2 HTS platform can effectively detect bioactivated and bioinactivated ER compounds and provides new positive and negative reference compounds for metabolism-dependent ER assays. This abstract does not necessarily reflect the policy of the US EPA.

2474 Development and Characterization of an In Vitro Human iPSC-Derived Neuropheroid Model

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Multiple approaches have recently been described to generate in vitro neural organoid models from human induced pluripotent stem cells (iPSC). While these models are effective at recapitulating many features of human fetal brain development, the processes used to generate them are not amenable to large-scale production and high-throughput applications. The goal of this exploratory research is to develop a scalable and reproducible in vitro, 3-dimensional neuropheroid model that may be used for high-throughput applications assessing neurodevelopmental toxicity (DNT) and drug development. Methods: Neuropheroids were generated by seeding iPSC-derived neural progenitor cells (NPCs) in ultra-low adhesion multi-well plates. Neuronal differentiation was induced with medium containing BDNF and GDNF. Neuropheroids were maintained in culture for up to 8 weeks. Immunofluorescent analysis of neuropheroids was performed on frozen sections and gene expression was assessed using real-time quantitative PCR (qPCR). This approach yielded neuropheroids of consistent size over time, both within and across batches. Immunofluorescent analysis of frozen neuropheroid sections revealed widespread expression of neuronal markers MAP2 and βIII-tubulin, as well as VGLUT1, indicating presence of presynaptic glutamatergic specializations. qPCR analyses showed increased in expression of neuronal genes including MAP2 and synaptophysin over time, while expression of progenitor markers (SOX2, nestin) decreased. Neuropheroids of consistent size containing mature neurons were successfully generated using this approach. The model is easily scalable and can be adapted to either 96- or 384-well plate formats, allowing use in high-throughput applications. Further efforts will use this platform to establish assays measuring cellular toxicity and processes modeling DNT. Neuropheroid complexity will also be improved by the incorporation of additional cell types such as astrocytes and microglia.

2475 3D-Bioprinting Microtissues with Human 1T1 Urothelial Cells Exposed to Diuron and Its Metabolites


Three-dimensional (3D) cell culturing has been used as an alternative method to toxicological assays that use laboratory animals, including for pesticides hazard evaluation. Exposure to the herbicide Diuron has been associated with rat urothelial proliferative lesions and tumors. This study aimed to standardize the 3D bioprinting technique to culture 1T1 urothelial cells, donated by Samuel M. Cohen, UNMC, Omaha-NE, as microtissues, applying both methods. The bioprinting protocol for and 14 compounds reported to be unaffected by metabolism was designed for a training set evaluating the AIME approach (Pinto et al. DOI: 10.1021/acs.chemrestox.6b00079). All 48 compounds, plus assay-specific controls selected from OECD test guidelines, were screened using the AIME-VM7Luc4E2 coupled assay. AIME lids were prepared with either phenobarbital/5,6-benzo[alpha]pyrene-induced rat hepatic S9 (metabolism positive) or algalate-only (metabolism negative) microspheres and immediately added to 96-well plates containing test compounds in 50-point concentration-response in cell culture medium supplemented with an NADPH regeneration system. Following a 2-hour incubation period, the conditioned medium was transferred to estragon-stripped VM7Luc4E2 cells and incubated for 24 hours to evaluate ER transactivation and cell viability. The resulting scores for the AIME-coupled assay were 0.71 and 0.91 for metabolism positive and metabolism negative treatments, respectively. To capture efficacy and potency shifts resulting from metabolic activity, the difference in the fitted area under the curve for corresponding treatment groups (+/− metabolism) was calculated. Bioactivation was identified for 21 compounds while bioinactivation was observed for 18 compounds. Half-maximal active concentrations (AC₅₀) for the majority of the hits was below the compound-dependent cytotoxicity cutoff, indicating relevant bioactivity. These data demonstrate the metabolism-coupled AIME-VM7Luc4E2 HTS platform can effectively detect bioactivated and bioinactivated ER compounds and provides new positive and negative reference compounds for metabolism-dependent ER assays. This abstract does not necessarily reflect the policy of the US EPA.

2019 SOT Annual Meeting
Using ViaLight Plus Cell Proliferation and Cytotoxicity BioAssay to Analyze Cell Viability in a 3D Breast Model

Due to increasing regulations surrounding in vivo studies, complex in vitro models are necessary for pre-screening compounds for toxicity and efficacy prior to preclinical studies. 3D cell culture models provide a more physiologically relevant system for evaluating drug compounds, but the dense, tissue-like matrix of the model can make evaluation of various endpoints difficult. One such endpoint that can be challenging to measure in 3D is cell number and viability. Which is critical in understanding the efficacy of cytotoxic or cytoprotective compounds. This work describes how to measure cell viability, and compare cell numbers, in a 3D hydrogel cell culture with the ViaLight™ Plus Cell Proliferation and Cytotoxicity BioAssay. The ViaLight™ assay is based on the biosensor luminescent detection of cellular ATP as a measure of cell viability thus determining cell numbers. We constructed a breast model using the RAFT™ 3D Cell Culture System. Our model is a co-culture of human mammary fibroblasts (HMF) embedded in the RAFT™ Collagen matrix, and human mammary epithelial cells (HMEC) overlaying on top of the matrix, to model the interaction of breast and stromal cells in vivo. Cell morphology was determined using microscopy and results demonstrate that HMEC are 90% confluent and HMF are fully stretched in the matrix after 3 days in culture. Cell proliferation was quantified with the ViaLight™ assay, which correlates cell number with increasing biosensor luminescence of cellular ATP. Data from this assay demonstrates an increase in cell number from both RAFT™ mono-culture and co-culture models from Day 3. On day 3, we added PIK-75 (Selleckchem S1205) to induce cell cycle arrest, which resulted in decreased biosensor luminescence from Day 4 to Day 8 and demonstrated the capability of the system to detect changes in cell numbers, and, in this case, cell loss. We demonstrate the feasibility of co-culturing HMF and HMEC, the RAFT™ system, as a 3D mammary acini model. Furthermore, the ViaLight™ assay can be used to measure changes in cell number in the 3D model after treatment with an anti-cancer drug. This assay, and its ability to evaluate cell number in a 3D model, can be used to bridge the gap between 2D HTS and preclinical studies by providing more physiologically-relevant data to better predict the in vivo efficacy, toxicity, and dosage of the drug candidates identified in HTS, thereby reducing the burden of subsequent studies in drug discovery.

Identification of Risk Factors for Breast Cancer Using Engineered In Vitro Techniques

Identification of Risk Factors for Breast Cancer by Activation of Fibroblasts and Thus Secretion of Collagen I

Our preliminary studies show that under acute ROS exposure, epithelial cells show loss of polarity and altered nuclear morphology that controls chromatin arrangement and differentiation. Our central hypothesis is that microenvironmental stress in synergy with altered matrix stiffness leads to increased breast cancer risk. To test our hypothesis we use a 3D cell culture model that consists of a co-culture of human mammary epithelial HMT-3522 S1 and human mammary fibroblasts HMs322 HTERT cells. Acute OS was induced by exposure to 250 μM H2O2 for four hours and chronic OS by treating the cells with 25 μM H2O2 for 10 days. Immunostaining with DAPI for nucleus and phalloidin for actin cytoskeleton, changes in phenotype of cells under ROS and altered stiffness were assessed. To test the effect of matrix stiffness on nuclear morphology S1 cells were cultured on collagen I coated with laminin 111 to induce differentiation, and fibroblasts were embedded in collagen I matrix of normal and at-risk stiffness in vivo. Increased stiffness leads to significantly less circular nuclei in both S1 cells and fibroblasts. Fibroblasts show a loss of spindly phenotype under acute ROS. Chronic ROS leads to less circular and bigger nuclei in S1 cells similar to as observed when stiffness of the matrix was increased suggesting that both ROS and matrix stiffness impact epithelial cells and fibroblasts independently. To understand whether these two micro-environmental features synergistically exacerbate the risk of cancer under in vivo conditions, a tissue chip will be developed where S1 cells will be cultured on top of collagen in collagen I matrix and co-cultured with HMs in hemichannels on paper to mimic ductal geometry with holes (to enable the epithelium to sense the stiffness underneath) and placed on top of collagen I embedded fibroblasts.

A Multiple Organ Integrated In Vitro Model for Studying Repetitive Dose Toxicity

Repetitive dose animal toxicology studies are still required within many regulatory frameworks for human risk assessments, but they are costly, time-consuming, and questionable validity, and ultimately limited in the information they can provide. Replacement of these in vivo studies with an adequate in vitro approach is a major technological challenge. Here, we present the first ever optimized, multiple organ, integrated in vitro system with simulated blood flow capable of addressing human systemic toxicity risk. Two well studied compounds, acetaminophen (APAP) and cyclohexamide (CYHex) were tested as a proof of concept. The compounds were evaluated in an integrated in vitro platform (HuDMOP™) that links human 3D intestine (EpiIntestinal™, MatTek), breast cancer by activation of fibroblasts and thus secretion of collagen I. ROS could also influence mammographic density, another risk factor for breast cancer, known to play a role in breast cancer risk and when not cleared by antioxidants mechanisms in the breast tissue-chip will be developed where S1 cells will be cultured on top of collagen I matrix and co-cultured with HMs in hemichannels on paper to mimic ductal geometry with holes (to enable the epithelium to sense the stiffness underneath) and placed on top of collagen I embedded fibroblasts.
The majority of pharmaceutical drug candidates that enter clinical trials ultimately fail safety and efficacy testing because of drug induced vascular injury (DIVI). The mechanisms of injury are often difficult to decipher as the drug metabolism, the toxic dose, and the affected vascular bed are species dependent. Several in vitro tools have been developed to evaluate DIVI, but the mechanisms and biomarkers for DIVI remain unclear. DIVI has been assessed in an engineered microfluidic model of a rat small artery. Our device consists of a monolayer of endothelial cells (ECs) and multiple layers of smooth muscle cells (SMCs), separated by a novel semi-permeable membrane. Media is circulated through each device-bioreactor system by a micro peristaltic pump. The engineered endothelial cell layer is matured and compounds are recirculated under shear stress. Vascular injury is characterized by leakage of FITC-Dextran (FD70) and by histological changes in the vascular wall. ECM bilayers in these microfluidic devices and static transwells were challenged with drugs known to induce DIVI in rats (fenoldopam, midodrine and nicorandil) as well as a negative control (yohimbine). Each drug was tested at Cmax, 10X Cmax, and 100X Cmax at the following rates: 0, 67, 91% fenoldopam; 0, 50, 100% midodrine and nicorandil. Drug exposure with FD70 leakage also showed histopathological changes. However, no FD70 leakage and histopathological changes were observed in static transwells tested with the same drugs. In conclusion, these results demonstrate a dose-dependent correlation between DIVI observed in our in vitro microfluidic device and that observed during in vivo rat testing. Furthermore, the pressure and shear parameters can be modified within our model, allowing our engineered vascular device to effectively assess both the impacts of drug toxicity and hemodynamic factors on DIVI.

Assessment of corneal drug penetration is crucial for development of effective ophthalmic medicines. Current studies utilize excised animal corneas that are not suitable for rapid drug screening, have poor species extrapolation and standardization. Physiologically relevant, human-based in vitro models for development of new ophthalmics are needed. This study evaluated the utility of an in vitro reconstructed tissue model (EpiCorneal) to study ophthalmic drug delivery. The model contains normal human corneal epithelial cells that grow at the air-liquid interface and develop a tight barrier (TEER 1000 ±250 Ω*cm). The performance of the reconstructed corneal tissues was demonstrated using formulations of known antiglaucoma drugs. The effect on drug absorption, tissue viability (MTT assay) and integrity (TEER, LY leakage) was investigated after 0.5h and 2h treatments and compared to the tissues treated with the Krebs-Ringer buffer (KRB). Steady-state flux reached by latanoprost (LAT) in commercial formulation and LAT in KRB was 3.47 and 2.04 nmol/(cm²h), respectively. Flux for bimatoprost (BIM) in commercial formulation (Lumigan) and BIM in KRB was 18.33 and 3.43 nmol/(cm²h), and Papp was 5.48x10⁻⁵ and 1.47x10⁻⁵ cm/sec, respectively. Flux for bimatoprost (BIM) in commercial formulation (Lumigan) and BIM in KRB was 18.33 and 3.43 nmol/(cm²h), and Papp was 5.48x10⁻⁵ and 1.47x10⁻⁵ cm/sec, respectively. Flux reached after application of free acid metabolites of LAT/BIM in KRB was 0.18.50.53 ml/m² (cm²/h), and Papp was 1.22x10⁻⁵ 1.85x10⁻⁵ cm/sec, respectively. Incubation with commercial LAT/Lumigan for 0.5h resulted in a 1.8/3.2-fold increase in tissue leakage and a decline in TEER to 42.2/ 44.1%; additionally, tissue viability declined to 71.0/74.2% after 2h. All other eye drop formulations did not have an impact after 0.5h. However, 2h exposure reduced TEER to 65.7%/77.8% for LAT/BIM. Both commercial formulations contained 0.02% Benzalkonium Chloride (BAC) as a preservative. By itself BAC at concentrations 0.005% and 0.01% in KRB did not have an effect on 3D tissues; however, at 0.02% it reduced TEER to 51.6% and 0.04% - reduced TEER to 18.3%, increased LY leakage (3.5%), and reduced viability to 43.8%. In summary, the EpiCorneal model demonstrated high predictive capabilities for drug bioavailability/biocompatibility of various known ophthalmic formulations/exciipients. The tissue model may be useful for evaluation of corneal drug permeability and safety during the development of new ophthalmics.
The HuDMOP™ system is an integrated meso-scale multiple organ culture plate that may be used to predict human pharmacokinetics (PK) and pharmacodynamics (PD). Here we assessed 2-phenoxyethanol (PE) PK and PD in this system. PE is commonly used in cosmetics, personal care products, and pharmaceuticals. PE is well characterized and is metabolized to 2-phenoxyacetic acid (PAA) in the body. The first HuDMOP™ setup utilized conditions of Tumult in testis (Epilintestine™), MatTek and primary human hepatocytes in sandwich culture (HHSC) linked via simulated blood system. In the second setup, intestine was linked to HHSC and kidney cells (primary human proximal tubule epithelial cells (HRPTEC)) via a simulated blood system. PE solutions (100 µL of 0.5, 1, 5, and 50 mM) were applied to the apical side of the intestinal model. Samples were collected from the basolateral intestine media, liver media, kidney media, and the simulated blood (perfusion) at 0, 1, 2, 4, 6 and 24 hr. PE and PAA were measured in each sample by LC-MS/MS. Maximum concentration of PE in the intestinal basolateral media and perfusate was reached at 1-4 hr and was dose dependent. PE in the liver media was quantifiable at 2.4 hr, occurring more rapidly with higher doses. PE was also observed in the kidney media by 4 hr while PAA was detected at 24 hr in liver media and in the perfusate. The time to maximum PE and PAA concentration, and movement of PE and PAA through both setups, was dose and time dependent. Tissue health was determined by measuring Ki-67, an indicator of proliferation. No substantial toxicity was measured over the 24 hr period. Finally, expression of interleukin-8 (IL-8), tumor necrosis factor alpha (TNFa), cytochrome P450s 1A1 (CYP1A1) and 3A4 (CYP3A44) were assessed by qPCR in all tissue types. No changes in expression were observed in the liver, and kidney, however a slight induction of CYP3A4 (2-fold) and IL-8 (2.7-fold) and 2.7-fold, respectively were observed in the intestinal tissue. These results suggest no substantial irritation response to PE or PAA which fits the known toxicological profile of PE at the concentrations assessed. Additionally, the PE Tmax observed here (1-4 hr) matches known in vivo results (1 hr). The observed PE Cmax, while dose dependent, appeared to correlate with the concentration in vivo results as well. These data indicate that the HuDMOP™ model may be an effective platform to properly model PE and predict PK and PD in humans.
Automated approaches to perform high throughput toxicity screening often employ microscopy to capture photomicrographs from multi-well cell culture plates (e.g., 96- and 384-well) generating thousands of images that require time-consuming manual analysis. Moreover, human interpretation of cell culture morphologies remains an interpretative activity, analogous to the practice of clinical pathology, and remains a significant barrier in regulatory adoption of these methods. To automate this subjective and time-consuming manual process, we have developed a method that uses deep learning to automatically classify digital assay images. In the preliminary phase of our efforts, we have trained a convolutional neural network (CNN) to classify assay images into healthy (comparable to vehicle controls) and altered (not comparable to vehicle controls). The dataset comprised 282 high-resolution (1392x1036 pixels) assay images from differentiated cultures of HepaRG cells, annotated for altered and healthy classes by two expert annotators. Inter-annotator agreement was 0.61 (Cohen’s kappa measure); we considered only those images which were agreed upon by both annotators. Traditional feature-engineering methods coupled with shallow classifiers (e.g. random forest, SVM) resulted in accuracies ranging from 50-80%. To improve these results, we developed three deep-learning based approaches: 1) pretrained CNN as fixed-feature extractor; 2) fine-tuning of pre-trained models; 3) training a CNN from scratch. Each image was divided into an n x n square grid where n=16. Each patch was annotated as the entire image leading to an n²-fold increase in the size of the dataset. Splitting the assay image into patches also has the advantage of detecting focal damage that often radiated out over time. The predicted label to use for each image was decided by adopting two different methods of aggregation across patches: 1) majority vote, and 2) mean predicted probability. All three deep-learning methods scored ~95% five-fold cross-validation accuracy when classifying assay patches into healthy and altered classes, with approach Number 3 performing the best (97.3%). The CNN trained from scratch had 96.7% accuracy on a ‘hold-out’ test set of 30 images. These results clearly demonstrate that deep-learning based image classification of cell toxicity can yield highly accurate results in small culture wells that is highly automated and reproducible for high throughput screening in toxicology.
Classifying Polycyclic Aromatic Hydrocarbons by Carcinogenic Potential Using Human Systems Biology Data in a Pathway-Based Interactive Model

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Current US EPA framework for human carcinogenic risk assessment of polycyclic aromatic hydrocarbons (PAHs) uses the relative potency factor (RPF) approach, a component-based approach that estimates RFPs for single PAHs using mouse in vivo tumor data. Key assumptions of this approach are that PAH-induced toxicity in mouse in vivo is representative of human PAH toxicity, and that mechanisms of toxicity are similar to benzo[a]pyrene (BAP), the reference carcinogen. To explore the validity of these assumptions, we evaluated differences in global gene expression of PAH-treated human bronchial epithelial cell (HBEC) model, and tested predictive model performance in PAH/PAH mixture classification by feeding the model with mouse in vivo or human in vitro data. HBEC were treated for 48 hrs with the following: benzo[a]pyrene (BAP), dibenzof[cd]chrysene (DBC), pyrene (PYR), coal tar extract (CTE), phenanthrene, benzo[a]anthracene, and simulated air mixture of PAHs representative of components in extracts from Beijing. Previously, a predictive model integrated 4 pathways linking 12 hr in vivo mouse dermal exposures to in vivo mouse tumor outcome. Bayesian integration of these pathways (apoptosis, interferon gamma signaling, response to DNA damage, and cell response to chemical stimulus) using k-nearest neighbors algorithm with LOOCV successfully classified treatments into low, moderate, and high tumorigenic potential. Model performance was tested by seeding with HBEC data for a subset of PAH treatments previously used in the mouse to develop the model, and successfully classified BAP, DBC, PYR, and CTE with a classification accuracy of 0.92. However, the model performed poorly (<0.30) when additional treatments with potency ranges that were not tested in mice were seeded into the model. While the model supported concordance between mouse and human in tested chemicals, it required pathway modification to better reflect a broader range of PAH potencies compared to BAP. This preliminary model is a valuable first step towards using systems biology data to assess carcinogenic potential, and supports using mechanistic data to inform whole mixture carcinogenic risk assessments of complex PAH mixtures.

An Integrated Approach for Animal-Free Genotoxicity Testing: In Vitro and In Silico Evaluation and Mode-of-Action Classification


New alternative approaches (NAMs) for the rapid and cost-effective screening of genotoxicity are promising potential replacements for traditional tests. ILS has developed a workflow to evaluate chemicals for possible genotoxicity potential as determined by in silico predictive modeling and human-relevant in vitro testing. High-throughput mammalian cell-based genotoxicity screening assays in human relevant TK6 cells were performed using a validated MultiFlow® DNA damage - p53, yH2AX, Phospho-Histone H3 kit which informs on genotoxicity and potential mode-of-action. Chemicals were also evaluated using 14 CASE Ultra-Software (MultiCase, Inc.) for quantitative structure-activity relationship (QSAR) genotoxicity predictions, including assessment of an overall genotoxicity prediction as well as consensus predictions from 4 models for carcinogenicity and 10 models for mutagenicity. For this proof-of-concept study, food-use chemicals were evaluated, including direct food additives and flavoring compounds. Results highlight that the MultiCase predictive modeling dataset rarely contained similar chemistries as seen in many of the food-use chemicals, namely flavorings. Since the predictive modeling approach relies on QSAR, which is based on chemical structure analog similarity, this is a serious limitation. Conversely, the in vitro approach are more amenable to screening compounds with a variety of structure types. Overall, food-use compounds were correctly identified as negative for genotoxicity based on in silico and in vitro data. While this reflects high accuracy, it also limits the capacity to evaluate performance metrics due to a heavy bias toward the resulting dataset’s abundance of true negative findings with very few positive chemicals. This study demonstrates effective NAM use for evaluating genotoxicity, emphasizes the need for study design considerations when considering predictive modeling software, and exemplifies the impact of domain of applicability limitations when considering alternative approaches. Ultimately, this integrated approach, leveraging both in vitro assays and in silico predictions, can be applied to any class of chemicals to effectively screen for genotoxicity potential.

In Silico Acute Toxicity Protocols and Models

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In silico toxicology is an important alternative approach to animal testing that can provide many benefits. Whole computational approaches can quickly calculate a prediction, the process of selecting and acquiring models, performing an expert review, integrating experimental data and model results, and documenting conclusions and uncertainties can be time-consuming and difficult to repeat. It is also challenging to defend the results, primarily due to a lack of published procedures for performing an in silico assessment. To support the development of such protocols, a SS-member international cross-industry consortium has been assembled that includes representatives from international regulatory agencies and government research laboratories in the United States, Canada, Japan and Europe, as well as large companies from various industrial sectors (e.g., pharmaceutical, food, cosmetics, environmental chemicals), academic groups and other stakeholders. The protocols will ensure any in silico assessments are performed in a consistent, repeatable, well-documented and defendable manner to support their broader acceptance. To support the implementation of the acute toxicity protocol, this work presents the development of a series of in silico models to predict acute toxicity based on GHS categories from rat oral studies. A battery of structural fragment-based models were used to predict these categories. Each model predicts a range of contiguous values such as the probability that a chemical is in GHS category 1 or 2. The cross-validated performance of these models ranged from 70.3% - 78.6% sensitivity and from 67.9% - 97.2% specificity.

The Delaney Clause, from 1958 to 2019: Making the Model Relevant

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The Delaney Clause of the Federal Food, Drug, and Cosmetic Act, named after Congressman Jim Delaney, was enacted in 1958 because he was worried that potentially harmful chemicals from food were responsible for causing cancer. It states that “no additive shall be deemed to be safe if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal.” The US FDA and US EPA are the two federal agencies charged with implementing this clause. It has been 60 years and significant advances in cancer research have elucidated the causes and mechanisms by which chemicals induce cancer. Advancements in analytical methodologies are allowing for accurate and progressively lower detection limits, resulting in detection of trace amounts of chemicals. Based on the current scientific knowledge, there is a need to look at the Delaney Clause from a different lens and make it more relevant. As a scientific community, we are committed to improving public health by promoting the development and utilization of appropriate and relevant science in risk assessment and regulatory decision-making. The objective of this roundtable session is to provide a balanced discussion and propose a path forward. The presenters in the session will provide (1) a historical overview of the scientific advances in cancer research since the 1950s, (2) the role of the Delaney Clause from a different lens and make it more relevant based on 21st-century science.
process has been undergoing “fixes” in real time as attempts are made to navigate the statutory requirements for new chemicals without undue delays hindering innovation. More recently, discussions about data transparency for information used by US EPA in its regulatory decisions have become a prominent part of the narrative. In addition, there are extensive efforts around how nonanimal approaches can be used to fill data and information needs. There are both challenges and opportunities that must be addressed. Each of the three presentations will provide the perspective of the groups they represent on how these challenges and opportunities can be addressed to achieve progress toward an improved chemicals management process in the US.

2499 Differing Effects of Perfluorooctanesulfonic Acid-Induced Hepatic Steatosis: Influence of Diet Type and Timing for Hepatic Steatosis Outcomes

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It is estimated that 20-30% of the population present with non-alcoholic fatty liver disease (NAFLD) in the United States alone. The contribution of environmental factors as risk factors for fatty liver disease is not well known. Per- and polyfluoroalkyl substances (PFASs) are a group of man-made chemicals that can be found in food packaging, stain repellent fabrics, and non-stick products. PFOS, PFOA, PFNA, and PFHxS are known to have multiple adverse liver effects in rodents and have been detected in human liver biopsy samples. The aim of our studies have been to evaluate the interaction between diet and PFOS exposure to determine whether they are an additive risk factor for hepatic steatosis and could augment hepatic lipid content and biomarkers associated with NAFLD. This presentation will summarize the findings of several new studies we have conducted with various diet combinations, such as a high fat diet (HFD) versus a 45% moderate fat diet (MFD), and diets containing high fructose, as well as shifts in timing of diet relative to PFOS exposure (0.0003% in diet). Endpoints related to hepatic steatosis, gene expression, as well as relative changes in protein abundance in liver using MS-SWATH will be presented. For example, hepatic steatosis was augmented in adult male mice that had an obesity and diabetes phenotype established after 8 weeks of HFD feeding. This was associated with increased expression of Cyp4a14 and other genes involved in lipid deposition/metabolism (C3D6, Fabp4, Slc271a). In contrast, simultaneously starting a MFD and PFOS exposure in early adulthood appeared to be protective in mice. Both studies demonstrated induction of genes related to the antioxidant response and the Nuclear Factor E2 Related Factor 2 (Nrf2) and Constitutive Androstane Receptor (Car) pathways. Use of novel antioxidant response element transgenic reporter mice will be also presented to determine whether PFOS exposure influences Nrf2 activation in liver, as well as other tissues. Overall, Cyp4a14 and C3D6 were consistently upregulated in liver after low dose PFOS treatment in the various models. Timing of HFD introduction may be an important consideration for the influence of various PFASs on the development of hepatic steatosis, and mechanisms that explore the impact of a HFD on hepatic PFOS levels and signaling must be considered.

2500 The Progression of Steatosis to Steatohepatitis with Fibrosis following Aryl Hydrocarbon Receptor (AhR) Activation

T. Zacharewski. Michigan State University, East Lansing, MI.

Aryl hydrocarbon receptor (AhR) activation by persistent environmental contaminants elicits a broad spectrum of adaptive and adverse responses. This includes a non-alcoholic fatty liver disease (NAFLD)-like syndrome, involving the dose-dependent progression of benign and reversible steatosis to steatohepatitis with fibrosis. NAFLD is a risk factor for more complex diseases such as metabolic syndrome, diabetes, cardiovascular disease, hepatocellular carcinoma and is also emerging as a leading cause of liver transplants. The integration of complementary RNA-Seq, ChIP-Seq and metabolomics data with histopathology suggests the dose-dependent activation of AhR by TCDD programs multiple metabolic pathways, consistent with the multiple-hit hypothesis describing NAFLD development and progression. For example, TCDD increased fatty acid transport, repressed \( \beta \)-oxidation and inhibited lipid efflux resulting in hepatic steatosis. In addition, the hepatic accumulation of free fatty acids and the induction of cytochrome P450s, increased ROS levels and lipid peroxidation leading to extracellular matrix remodeling and the deposition of collagen along portal tracts. In response, hepatocytes mount a Warburg-like response involving the reprogramming of central carbon metabolism to support ROS defenses. More specifically, TCDD induced pyruvate kinase muscle isoform 2 (Pkm2) which has reduced catalytic activity compared to Pkm1. Pkm2 induction facilitates the accumulation of upstream intermediates that are redirected to the pentose phosphate pathway and the serine/glycine biosynthetic pathway to produce NADPH and intermediates in support of glutathione biosynthesis and recycling. AhR activation by TCDD also induced a de-differentiated hepatocyte phenotype, as suggested by the repression of amino acid metabolism and loss of typical liver-specific and sexually dimorphic gene expression patterns that further compromise liver function. This presentation will present new data demonstrating the development of environmental factors in NAFLD progression and a more comprehensive understanding of key factors involved in the progression of NAFLD. Taken together, information from this session has implications for risk assessment in affected populations and for defining mechanisms and potential biomarkers of liver damage.
2501 Toxicant-Associated Steatohepatitis: Clinical and Translational Studies
M. Cave. University of Louisville, Louisville, KY.

Since the initial description of toxicant associated steatohepatitis (TASH) in 2010, epidemiologic studies have demonstrated associations between pollutant exposures and fatty liver disease. This session reviews the progress and challenges facing liver disease cohort studies. TASH was initially described in vinyl chloride workers who had a high prevalence of biopsy-proven steatohepatitis. While affected workers had normal liver enzymes, the hepatocellular necrosis biomarker, cytokeratin 18 M65, was elevated. Altered cytokines and insulin resistance were identified as possible mechanisms. A subsequent serum metabolomics study demonstrated a unique metabolite signature in vinyl chloride workers. Altered AMPK signaling and mitochondrial dysfunction were predicted. These mechanisms have subsequently been confirmed in animal exposure models. We then performed the first exposome-wide analysis investigating pollutants and fatty liver disease using NHANES. PCBs were dose-dependently associated with increased odds for suspected fatty liver disease. These results have been confirmed by other groups. It is challenging to perform liver biopsies in environmental cohort studies, and liver enzymes may be normal in TASH. We are currently addressing this challenge with biomarker panels (e.g., cytokotyosis markers, adipokineyons, and microRNAs). The Anniston Community Health Survey (ACHS) is a residential cohort with high rates of obesity and PCB exposures. In ACHS, there was a high prevalence of liver disease associated with hepatocellular necrosis, insulin resistance, cytokine elevation, and PCB exposures consistent with TASH. Liquid liver biopsy was performed in a subset of participants using a targeted serum microRNA panel. Pathway analysis confirmed steatohepatitis in participants previously categorized as having TASH. In the subset of subjects with liver disease participating in the ACHS-II re-contact study, dioxins and dioxin-like chemical exposures (including non-ortho PCBs) were associated with altered serologic biomarkers of hepatic intermediary metabolism, inflammation, fibrosis, and function. These mechanisms have been confirmed in animal models of PCB or vinyl chloride exposure. The clinical and translational studies demonstrate that pollutant exposures may cause steatosis or drive the progression of diet-induced steatosis to more advanced liver disease.

2502 Integrated ‘Omic Approaches to Toxicity Assessments
J. Rager. University of North Carolina at Chapel Hill, Chapel Hill, TX.

Recent advances surrounding assay and sequencing-based technologies have increased the feasibility of multi-omics research, wherein two or more ‘omic profiles (e.g., genomics, transcriptomics, epigenomics, metabolomics, and proteomics) are integrated and evaluated to further understand molecular mediators of biological function and cellular health. Multi-omic analysis strategies rely upon the joint analysis of multiple data types, yielding toxicity responses that may not have been identified given the analysis of only one ‘omic endpoint. Integrated analyses provide insights into how the features of the ‘omics interact through biological networks, resulting in an integrated systems level understanding of toxicity. The utilization of multi-omic strategies presents the opportunity to elucidate hierarchical processes in complex systems that can further substantiate mechanisms of action linking chemical exposures to disease, which ultimately aids in the accurate assessment of chemical risk and overall protection of public health. Integrated ‘omics strategies are also employed to understand mechanisms of action linking pharmaceuticals to health outcomes. However, methods and associated databases that can be leveraged to integrate multi-omic response signatures that result in findings useful for drug development and chemical assessments are still under development, with current limitations that require further research. This session will address this growing research area by discussing multi-omic data integration tools, data reduction methods, dose-response modeling, toxicity predictions based on machine learning algorithms, and systems level analyses to elucidate molecular pathways involved in disease etiology. Case studies also will be discussed to provide examples of how multi-omic response signatures can inform toxicity assessments relevant to environmental regulation and the chemical/pharmaceutical industries.

2503 Computational Tools for Multi-‘Omic Integration
K. Uppal. Emory University, Atlanta, GA. Sponsor: J. Rager

Current approaches for multi-omics data integration can be classified into: data-driven, pathway-driven, and literature-driven. The presentation will first give a comparative overview of the three approaches with examples of available bioinformatics tools for each category. The last half of the talk will focus on xMwas, a multi-omic data integration tool, which is available both as a web-based application and R package. The software allows network-based integrative analysis of up to four datasets generated from the same subjects. It features additional capabilities such as detection of modules (clusters) in integrative networks and differential centrality analysis.

2504 An Integrated ‘Omic Dose-Response Assessment from Short-Term In Vivo Studies of Two Aromatics Phosphate Flame Retardants
S. S. Auerbach. NIEHS/NTP, Research Triangle Park, NC.

Aromatic phosphate (AP) flame retardants are inhibitors of several esterases with a diverse collection of substrates. We have performed dose-response transcriptomics and serum metabolomics following a repeated dose study in male rats exposed to two different APs. Dose-sensitive integration of the transcriptomics, metabolomics, clinical chemistry and organ weights from the first study of triphenyl phosphate (TPHP) identified a novel relationship between changes in liver weight, cholesterol, triglycerides, sphingolipids and gene expression associated with lipid and xenobiotic metabolism. Hub gene analysis indicated PXR/CAR signaling interacts with cholesterol metabolism (specifically esterase activity) signaling which is plausibly associated with changes in serum cholesterol and increases in liver weight. Replication of the integrated analysis findings was observed in the second AP study that employed isopropylated phenol phosphate (IPP), suggesting that these effects are likely consistent across the AP class.

2505 On-the-Fly Machine Learning to Predict Adverse Drug Reactions by ‘Omic Integration of Drug Properties
A. Ma’ayan. Icahn School of Medicine at Mount Sinai, New York, NY. Sponsor: J. Rager

By collecting and organizing knowledge about the properties of drugs and small molecules, including chemical structure, metabolomics, imaging, transcriptomics, known and predicted targets, and clinical indications and use, we can predict adverse events and side effects of clinically used drugs and experimental compounds with machine learning. A system will be presented where users can select the adverse event that they wish to predict, the data sets the wish to use for training, and the machine learning methods they wish to employ. After making those selections, the user will be presented with a complete report that lists the probability of the predicted adverse events and an evaluation of the quality of such predictions.

2506 Scientific and Regulatory Update in the Application of the 3Rs Principle in Chemical and Drug Development
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The 3Rs principle (3Rs), developed as a framework for humane research, has become embedded in animal use legislation. There remains ample opportunity to improve efficient usage of animals and to facilitate the 3Rs, with the availability of new in vitro/in silico technologies. Most toxicology studies are conducted to determine safe doses and a reasonable margin of safety. This facet is considered during pharmaceutical development. In case of nonproprietary pharmaceuticals wherein the toxicity of the active is known and the excipients are not novel or adhere to regulatory guidelines, the need to conduct in vivo studies is minimal. However, country regulatory requirements vary and repeat of studies may be necessary. At the global level, although there are ongoing discussions on the 3Rs, follow-up and implementation have not been rapid and many countries have a lot of catching up to do, especially in revamping their country regulation. This symposium session will address the following objectives: (1) to understand the need to conduct animal studies to address safety/efficacy and meet regulatory requirements for nonproprietary
pharmaceuticals; (2) to adopt in vitro, in chemico, and in silico approaches for skin sensitization; and (3) to comprehend human-relevant, non-animal methodologies to predict toxicity and provide a scientific underpinning for the use of read-across techniques.

Despite efforts to implement the 3Rs, large number of animals are still used for nonclinical research and testing. Nonclinical research is required for regulatory approval of pharmaceuticals, and most of these tests include animals. The US FDA has an abbreviated approval process for nonproprietary pharmaceutical products where no safety or efficacy data is required if the generic is comparable to the innovator product. In terms of active ingredient, strength, dosage form, route of administration, quality and performance characteristics, the European Union has implemented regulations that mandate use of alternative approaches whereas both China and Russia are working through the process of developing appropriate approaches to the implementation of the 3Rs in their countries. There are various options to effectively implement the 3Rs using in vitro alternative approaches, consideration of mutual acceptance of data, use of efficient study design, and harmonization of country regulations to name a few. Applying advances in the scientific thought process and state of the art technology to the development of sound alternative strategies can improve animal welfare and further reduce and replace animal use. In this symposium different strategies that have worked for effective implementation of the 3Rs in nonclinical development of nonproprietary pharmaceuticals will be illuminated. To better understand the need to conduct animal studies in generic drug development, several scenarios with relevant examples to address safety and regulatory requirement will be presented.

In a March 2016 open letter to stakeholders, former US EPA’s OPP Office Director, Jack HOUSENG, detailed OPP’s efforts to modernize the acute toxicity “six-pack” studies (acute oral, dermal, and inhalation, eye irritation, dermal irritation, and skin sensitization) and reduce animal testing. This letter committed OPP to explore opportunities to reduce barriers to alternative methods to animal testing and facilitate the use of OECD in vitro methods and computational approaches. In collaboration with NTP’s Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), industry and non-governmental organizations, the US EPA is making significant progress towards the adoption of integrated approaches to testing and assessment (IATA) and the reduction of the use of animals in acute toxicity testing. This presentation will provide an overview and update on progress to date and on-going collaborative projects, including a 2018 interim policy to adopt in vitro, in chemico, and in silico approaches for skin sensitization.

In Europe the principles of the 3Rs (refine, reduce, replace) are recognized at the level of policy and legislation. The Directive 2010/63/EU, which is a binding legal requirement for all member states of the European Union (EU), asks for considering the 3Rs systematically when considering animal experimentation. Advancing the “3Rs” through implementation of alternative approaches is not only benefitting animals but equally aimed at developing better and more predictive scientific tools to protect human and animal health and the environment. Most notable, amongst the legislations impacting the use of the 3Rs is the cosmetics regulation, which prohibits the testing of finished cosmetic products and cosmetic ingredients on animals. Also the European Chemicals Agency (ECHA) urges the use of non-animal methodologies for the purpose of chemical registration. Research in this field is sponsored by the European Commission through the flagship project EU-ToxRisk. This research initiative, comprising 36 partner labs, focuses on human-relevant, non-animal methodologies to predict toxicity. One major focus is to provide scientific foundation and guidance for the use of read-across (RAX) techniques. RAX makes toxicity predictions on unknown chemicals by linking them to known compounds. Structural and physicochemical similarity data will be linked in defined, quantifiable ways to adverse outcomes by incorporating mechanistic and toxicokinetic knowledge gained from new methods rooted in the 3Rs. Incorporation of similarities on the key event activation level (termed ‘biological RAX’) will advance the RAX concept to a robust process of assessing toxicological properties of chemicals that will be of practical regulatory use.

The determination of adversity, and a No-Observed-Adverse-Effect-Level (NOAEL), is often a fundamental component of the analysis of toxicological data. A NOAEL is often the basis for the determination of the First-In-Human (FIH) dose, although alternative methodologies like Highest Non-Severely Toxic Dose (HNSTD) and Minimum Anticipated Biological Effect Level (MABEL) have emerged to address specific situations. While the definition of adversity has often been a judgment call based on experience, a number of organizations have undertaken efforts to streamline and standardize the definitions and guidance on how to determine adversity. Study directors, pathologists, safety pharmacologists, preclinical drug development leads, and regulatory reviewers are among the key players involved in safety assessment who may have different perspectives and sometimes disagreements about determining adversity. The purpose of the session is to discuss the current thinking process behind the determination of adversity in nonclinical toxicology studies from multiple points of view. Moreover, all the speakers will also discuss their perspectives about dealing with unique situations in a toxicology study such as nonlinear vs. linear kinetics, sex differences in toxicokinetics and observed toxicity, misuse of historical control data, No-to-Partial Recovery of toxic effects, and on-target vs. off-target effects. Presenters will discuss these scenarios with the help of case studies wherever possible.
A study pathologist plays a critical role in categorizing adversity of gross histopathological lesions and clinical pathological changes. Sometimes, these findings may not be obvious to determine the adversity of the drug in the test species. In that scenario, how to handle the pathological findings and communicate those in the contributing scientists report in a way that helps Study Director to determine a NOAEL for the overall study? This presentation intends to give the attendees a pathologist’s perspective on determining adversity of anatomical and clinical pathological findings in toxicity studies. The presentation will discuss various factors such as severity, complete recovery versus partial recovery, functional significance, and sex differences in lesions; linear versus non-linear kinetics, and use and limitations of historical control data that are considered while evaluating adversity of histopathological findings. These factors will be discussed in the context of small and large molecule therapeutics. The challenges faced in communicating adversities in pathology reports will be addressed. This presentation will further discuss the handling of changes in the non-traditional clinical pathology biomarkers (Clinical Pathologist’s perspective) evaluated in GLP toxicology studies during adversity determination. The points in the presentation will be illustrated with the case studies.

A NOAEL is commonly used as a benchmark for FIH studies for many pharmaceuticals. The NOAEL is determined using the toxicology study data, as well as expert professional opinion of those data, in the toxicology package. Although the aggregate findings may be used between studies to inform the clinical team, the NOAEL should be determined for each study. Furthermore, the findings that inform the NOAEL may impact other aspects of the clinical trial (such as dose escalation or monitoring). Disagreements in the NOAEL (between regulatory and industry partners) are uncommon but may involve disagreement between the expert professional opinions/interpretations regarding adverse findings. Lastly, it is normally understood that for some immunomodulatory compounds, there may be challenges that may confound the use of a NOAEL for translation to human trials. Sponsors often rely on alternative methodologies (such as the MABEL) to set the first in human dose. This talk will discuss challenges to NOAEL selection and interpretation of the NOAEL into risk assessment during drug development with examples of some case studies.

The bisphenol class of chemicals includes over 20 analogues that have different structural, chemical, and biological activities. The primary analogue of interest in this class is Bisphenol A (BPA), a chemical widely utilized in plastics, epoxy resins, and other products. Most of the other analogues are not routinely used or used at high production levels, but exposure to some has led to evaluations to determine if there is any toxicological evidence for concern. The complications associated with toxicological evaluation of BPA may be attributed to the ubiquitous nature of this chemical. Carefully assessing the effects of this compound in animal studies is extremely difficult due to the requirement to control and monitor BPA from all external sources. It has therefore become essential to characterize dosing solutions, housing materials, and internal dose measurements to ensure that the animals are exposed to the levels of the chemical that the protocol dictates. While the analogues may not generate such a high level of attention, the primary question regarding their potential risks to humans is exposure levels. BPA is currently found at levels far exceeding any other analogue and its unique nature ensures that it is not easily replaced in a majority of products; however, there is evidence that the analogues are in use and information on their potential effects and endocrine activity will be essential if their use increases. An additional concern associated with assessing classes of compounds is determining effective methods to quickly and efficiently evaluate multiple analogues. High-throughput screening data and in vitro assays are not reliably "predicting" in vivo outcomes. This may be due to the use of assays not applicable for this class, focusing assessments to just the estrogen and androgen receptors, limitations with in vitro metabolism, or not recognizing that there needs to be a compromise when evaluating chemicals that are potential endocrine activators. Therefore, how to best generate reproducible and reliable data and understand the biology and/or chemistry of conflicts as they arise, as well as collecting routine internal measurements of the compound(s), may need to be a primary focus for those assessing chemicals in this group. The session will begin with an overview of the bisphenol class of chemicals and highlight its uses and products and then discuss testing of select analogues by different types of the in vitro assessments for this class of chemicals. In summary, the session will provide an overview of the bisphenol class of chemicals and the data that we have to date on the analogues, and discuss best methods for evaluating these compounds and the next steps in the assessment process. Disclaimer: This overview does not necessarily reflect any Agency opinions.

Bisphenol A is synthetic industrial chemical which has come under scrutiny in recent years. The chemical’s important industrial footprint and utility in various applications has led to a search for chemical substitutes. These substitutes are often structurally similar analogues to BPA and belong to a class of chemicals called bisphenols. To date, there are well over a dozen known analogues which range significantly in industrial utility and end uses. With a focus on the most common analogues, BPF (4,4′-methylene diphenol), BPS (4-hydroxyphenyl sulfone), and BPAP (4,4′-hexafluorodiphenyl ether), this session will provide a review of this class of chemistries along with the major uses. From epoxy lining to polycarbonate to even more obscure uses the attendee will learn why this class of chemistries is so useful, economically important, and hard to replace.
2517 Estrogenicity and Antiandrogenicity of Bisphenols: Uncertainties in Extrapolating from In Vitro Molecular Initiating Events (MIE) and In Vivo Key Events (KE) to Adverse Reproductive Outcomes

L. E. Gray, US EPA/ORD, Research Triangle Park, NC.

The Frank R. Launten Chemical Safety For The 21st Century Act requires that the US EPA minimize the use of vertebrate animals in testing chemicals by incorporating alternative test methods and employing a tiered screening and testing process that uses the results of screening-level tests or to inform the decision as to whether additional tests are necessary. These methods must "...provide information of equivalent or better scientific quality and relevance for assessing risks of injury to health or the environment of chemical substances or mixtures". This presentation will use the bisphenols as an example of the strengths and limitations of using the in vitro MIEs and in vivo KEs from the androgen (AR) and estrogen (ER) AOP to predict the adverse effects of chemicals on reproductive development and function. For the bisphenols, the in vitro ER activities are useful to identify potential estrogenicity since the analogues that are active in vitro typically are active in vivo. However, in vitro potency is not predictive of the potency in vivo. For example, BPS is weaker than is BPA in vitro while the reverse is true in vivo and BPA and BPF are equipotent in vitro, whereas BPAF is more potent in vivo, especially in mul-
tigenic species. A comparison of the in vitro AR antagonism with the in vivo effects of ~60 chemicals reveals that in vitro assays of AR antagonism have relatively high negative prediction rates, but low positive prediction rates. A significant percent of chemicals are AR antagonists in vitro but fail to induce effects that are pathognomonic for AR antagonism activity in the Hershberger assay or in multigenic tests. Based upon the above ob-
servations, the following tiered testing strategy is proposed for discussion. Chemicals should be grouped by class to determine if it is valid to utilize the in vitro data to prioritize in vivo studies. For the bisphenols, a positive in vitro ER signal could be used to prioritize analogues for short term in vitro ER screening assays. With evidence of AR antagonism discovered, bis-
phenols should not automatically trigger in vivo tests. In chemical classes like the bisphenols, where some of the in vitro data are of uncertain value, other non-animal methods and short term in vivo assays should be used to prioritize the need for more extensive testing in vivo.

2518 In Vitro Static and Dynamic Skin Metabolism Methods and Strategies to Address the Safety Assessment of Topically Applied Chemicals

A. Schepky, Beiersdorf AG, Hamburg, Germany.

Understanding a chemical's bioavailability, either locally or systemically, is important for predicting adverse effects and conducting a safety assessment. Metabolism was originally thought to be an inactivation or detoxification pathway for xenobiotics; however, today it is generally accepted that metabolism-mediated toxicity is important in regulatory toxicity. Therefore, all in vitro toxicity methods proposed for regulatory risk assessment should include careful consideration of metabolism-mediated toxicity. In addition to a detailed knowledge on metabolism, the biokinetics of the test chemicals and in the in vitro method setup will be vital for designing the most valuable and predictive integrated test strategies. The main exposure route of cosmetic ingredients is via the skin. However, once the parent compound or metabolites formed in the skin enter the systemic circulation, they can be further metabolized systemically (e.g., by the liver). In vivo skin models can help to predict the amount of parent and metabolite(s) formed locally in the skin and escaping first-pass skin metabolism. The session will present examples of metabolism of relevant chemicals in ex vivo human skin explants compared to 59 fractions from EpiSkin. Incubations with human liver 59 were also included to allow a comparison of liver metabolism to local skin metabolism. In addition, it was shown how the frequency and route of application of a chemical and its interaction with different organs can affect the kinetics and ratio of different metabolites formed systemically. For example, first pass skin effects have been reported for aromatic hair dyes as well as for several topically applied glucocorticoids. The route of exposure (topical vs. systemic application) may differentially de-
termines a chemical's effect on the liver. Qualitative as well as quantitative dif-
fences may be important aspects for risk assessment in cosmetic risk assess-
ment. The session will present how a 3D skin liver dynamic model can help us understand the interaction between skin and liver metabolism over extended and repeated exposure and test chemical-organ (e.g., sys-
temic) and how it affects systemic concentrations of both parent chemical and its metabolites. The threshold of toxicological concern (TTC) is a safety assessment tool that involves establishing a low-level exposure value, from known chemicals with curated toxicity data, below which there is a low probability of adverse effects. The concept of TTC as currently understood has evolved over the last 50 years and a logical next step in the con-
tinued evolution of TTC is to develop this concept further by converting the external NOAEL values to internal concentrations. This is especially relevant for chemicals with a low absorption, either via the oral or dermal routes, and thus relevant to the cosmetics, pharmaceutical, and chemistry industries. The development of iTTC thresholds would provide conservative hazard-based values that could be utilized in exposure-based safety assessments in the con-
text of (1) refinement of de minimis exposure levels for dermal exposures, (2) metabolite-based structure-activity relationship (SAR) assessments, (3) low-
level aggregate exposures from different dose routes, or (4) in vitro biological assays. The session will provide an update on the development of iTTCs and present case study examples for possible uses. In the absence of the use of animal studies in the cosmetics industry for safety assessment, in vitro alter-
natives and strategies to waive additional tests (e.g., the iTTC) are essential.

2519 Studying In Vitro Metabolism of Cosmetics Ingredients in Skin Explants or by Using Liver- or Skin-Derived S9 Sub-Cellular Fractions

C. Jacques Jamin, Pierre Fabre Dermotherapeutique, Toulouse, France.

Sponsor: A. Schepky

We evaluated 2 in vitro skin models for their suitability to investigate skin met-
abolism since this is important for assessing local toxicity and/or penetration of chemicals. In the ex vivo viable human skin explant model, chemicals are applied topically and distribution of the parent and its metabolites is mea-
sured in the skin and receptor fluid up to 48 h. Pig skin can be used as an alter-
native to human skin supplier limited. To identify specific metabolites, we compared the metabolism of 10 chemicals e.g. cosmetics (parabens, caffeine, resorcinol) and reference chemicals for toxicity assays (2-AAF, 2-chloroaniline in genotoxicity assays) using pig and human skin. The extent of metabolism was the same in pig and human skin. While some species differences in me-
tabolite formation were expected due to a lack of aryl SULT in pigs, there were other quantitative species differences in the relative amounts of com-
mon metabolites. Our data suggests that pig skin is metabolically competent and could be a useful tool to evaluate first-pass metabolism prior to testing in human-derived tissues. EpiSkin™ S9 fractions are short-term assays (up to 4 h) for higher throughput initial metabolism screening. We first confirmed whether this model provided similar information to the ex vivo skin explants. Incubations also included human liver 59 to compare dermal and hepatic me-
tabolism. Both in vitro skin models had functional Phase 1 and 2 enzymes, in-
ccluding CYPs. There was a good concordance between the models regarding the metabolite metabolism and formation. Not expected due to a lack of aryl SULT in pigs, there were differences in the metabolism of 2 chemicals were attributed to a lack of the appropriate cofactor (S9) and extensive protein binding in ex vivo skin. S9 incubations al-
lowed a ranking of chemicals into low, medium and high clearance chemicals and a comparison of rates of metabolism across chemicals with similar struc-
tures. S9 incubations enabled organ-specific metabolism to be studied under the same conditions. In conclusion, both skin models provide valuable information on the dermal metabolism of topically applied chemicals. The ex vivo skin model allows longer-term incubations and combines metabolism with skin penetration; whereas, EpiSkin S9 is a high throughput screening assay, providing an indication of the metabolic stability of a chemical in the skin.

2520 Understanding Chemical Metabolism in Skin and Liver Models over Extended and Repeated Exposure in a Multi-Organ Chip Device

J. Kuehnl, Beiersdorf AG, Hamburg, Germany. Sponsor: A. Schepky

Read-across approaches for different exposure scenarios require information on their respective bioavailability, since this may influence a chemical's sys-
temic effects. Cosmetic ingredients are mainly exposed to the skin; however, the parent compound or metabolites formed in the skin may be further me-
tabolized by the liver after entering the systemic circulation. Information on the first-pass effect of drugs is also important to assess - either in the skin for local skin therapeutics or in the liver for orally-administered drugs. To ad-
dress this, dynamic multi-organ-chip (MOC) based models aim to emulate the physiology of single organs and their interactions. These models may also help to interpret organ-specific toxicity e.g. toxicity in a target organ due to a metabolite formed in a different organ. Cosmetics Europe is evaluating the TissUse skin-liver MOC for its ability to add information on metabolism following single or repeat application via the topical and systemic routes. Initial proof-of-concept (POC) studies were performed on known xenobiotic metabolising enzyme (XMEs) inducers, permethrin and heparinoid. Data show that the route of exposure and the dosing frequency affect systemic concentra-
tions of parent chemicals and their metabolites; and chemical exposure al-
ters the XME profile of liver organoids over time, depending on the route and 2019 SOT Annual Meeting 361
frequency of exposure. To ensure changes in metabolite profiles are not due to toxicity, in-life measurements of organoid functionality and histology of the skin models are performed in parallel. The methodology was transferred to a second lab in which both POC chemicals were tested. Results for in-life measurements and metabolite profiles revealed excellent intra- and inter-laboratory reproducibility. Moreover, the changes in the kinetics profiles of the parent chemicals and metabolites were similar in both labs. In conclusion, our data support the use of the skin-liver MOC model to discriminate differences in the metabolite profile and kinetics of chemicals applied systemically and topically, after single or repeated application. These POC studies will help to support a biological concept that may help in read-across approaches for several repeated dose toxicity endpoints (with a focus on metabolism), especially in light of the Cosmetics Directive ban on in vivo studies.

2521 Internal Thresholds of Toxicological Concern as Tools for Assessment of Exposures via Oral and Dermal Routes

C. Ellison, Procter & Gamble Company, Cincinnati, OH. Sponsor: A. Scheppy.

The Threshold of Toxicological Concern (TTC) is an important risk assessment tool, which has evolved over the last 50 years, and establishes acceptable low-level exposure values to be applied to chemicals with limited toxicological data. This concept is based on external oral toxicity data, namely, the No-Observed-Adverse-Effect-Level (NOAEL), and as such, the corresponding threshold limits represent external exposures. With continued global interest in animal alternative methods for human safety risk assessment, one of the logical next steps in the continued evolution of TTC is to develop this concept further so that it is representative of internal exposures (i.e. a TTC based on plasma concentration). Therefore, as part of the Cosmetics Europe (CoSEu) Long Range Science Strategy (LRSS) research program, CoSEu has initiated a project that is working towards the development of internal TTCs (iTTC) that can be used for the human safety assessment. iTTC would provide threshold values that could be utilized in exposure-based safety assessments in the context of; (1) refinement of de minimis exposure levels for dermal exposures; (2) metabolism-based structure-activity relationship (SAR) assessments; (3) low level aggregate exposures from different dose routes; or (4) in vitro biological assays. We will provide an update on the development of iTTCs and present case study examples for possible uses.

2522 Innovation in Biomarker Qualification

S. Ramaiah, Pfizer, Inc., Cambridge, MA.

Biomarkers have become highly utilized tools in the drug development process. However, the unknown regulatory acceptance of biomarker data has made the routine incorporation of these tools and interpretation of their data unclear for some companies. Qualification of biomarkers removes this uncertainty for drug developers. This session will provide attendees with an overview of the US FDA Center for Drug Evaluation and Research (CDER) Biomarker Qualification Program (BQP) under the 21st Century Cures Act. US FDA BQP works with external stakeholders to develop biomarkers as drug development tools (DDT) to provide regulatory certainty regarding the acceptance of these tools. Presenters will discuss how the Critical Path Institute (C-Path), through their Predictive Safety Testing Consortium (PSTC), is working to advance qualification of novel safety biomarkers. Further, the newly formed TransBioLine (Translational Biomarker Pipeline) consortium of EFPiA pharmaceutical companies and leading European academic institutions and bioanalytical companies, which has been established to build on previous consortia work to advance regulatory qualification of new and emerging safety biomarkers, will be presented.

2523 21st-Century Cures Biomarker Qualification Program

C. Leptak, US FDA, White Oak, MD. Sponsor: J. Sauer

Qualified biomarkers can be powerful tools in advancing public health and medical outcomes by promoting efficiencies and innovation in drug development. As set forth in section 507 of the 21st Century Cures Act, the US Food & Drug Administration's Center for Drug Evaluation and Research (CDER) has enhanced its Biomarker Qualification Program (BQP) by working with external stakeholders to develop biomarkers as drug development tools (DDT). This presentation will review the BQP’s goals transparency provisions and resources, as well as the three submission phases needed for biomarker qualification, 1) the Letter of Intent, 2) the Qualification Plan, and 3) the Full Qualification Package. Finally, advancements made in the development of evidentiary criteria for the qualification of biomarkers over the past few years will be discussed.

2524 Critical Path Institute’s Predictive Safety Testing Consortium: Identifying Innovative Biomarkers to Accelerate Drug Development

J. Sauer, Critical Path Institute, Tucson, AZ.

Safety paradigms for testing new chemical entities have not changed in decades. Although companies have developed newer safety testing methods, these are not generally accepted by regulatory agencies as proof of safety. This is because methods are often different from company to company and in many cases have not been independently validated. The Critical Path Institute’s Predictive Safety Testing Consortium (PSTC) was formed in 2006 to serve as a catalyst in the identification of new drug development tools (DDT) by bringing together pharmaceutical companies in the pre-competitive space to share and validate innovative safety testing biomarkers. Validated biomarkers are then submitted for formal regulatory qualification. This presentation will address PSTC’s four-fold objectives in qualifying novel biomarkers: 1) to qualify and make DDTs publicly available to be used for a specific context of use in drug development, 2) to streamline drug development and review of regulatory applications, 3) to facilitate integration of qualified DDTs in regulatory review, and 4) to provide a framework for scientific collaboration to facilitate DDT development. Attendees will learn how PSTC leverages the expertise of multiple stakeholders including academic scientists, pharmaceutical and biomedical industry scientists, and regulatory authorities, to advance qualification of novel biomarkers. By working together as a consortium, industry leaders collaborate on safety biomarker projects by first identifying the drug development need; defining the content of use (COU) for the novel biomarker, identifying potential benefits and risks, and determining the evidence needed to support the COU based on the benefits and risks. Integrated within this presentation will be examples of novel safety biomarkers that have received regulatory approval for a specific COU based on the collaborative efforts of the consortium. In addition, current efforts will be shared to further advance innovation in qualification of novel biomarkers for hepatotoxicity, nephrotoxicity, testicular toxicity, and pancreatic, skeletal muscle and vascular injury will be highlighted. Attendees will also learn about PSTC’s biomarker data repository program as a new tool for advancing such qualifications.

2525 An Overview of the Innovative Medicines Initiative (IMI) TransBioLine Project

S. Ramaiah, Pfizer, Inc., Cambridge, MA.

Circulating safety biomarkers can improve the benefit to risk for patients and decrease attrition of drug candidates in industry via early detection and intervention. However, adverse event data related to a given adverse event for a specific organ specific biomarker discovery, development and qualification are of strong interest to drug developers, regulators and the broader scientific and patient communities. Additionally, the dual function of many safety biomarkers as diagnostic and efficacy biomarkers further augments their utility. Biomarker qualification requires innovative scientific approaches including bioanalytical platforms, and access to large sets of well annotated human samples and large international scientific partnerships that include industry, academic research, clinical investigators, bioanalytical companies and regulatory authorities. Recent success in biomarker qualification efforts achieved by the Critical Path Institute’s Predictive Safety Testing Consortium (PSTC) and the Foundations for the National Institutes of Health (FNIH) exemplifies innovative approaches and partnerships for formal qualification of emerging biomarkers. Also, the previous Innovative Medicines Institute (IMI) project SAFE-T consortium and the PSTC have generated several safety biomarker candidates that are under review by EMA, US FDA and PMDA. Despite these advances, significant safety biomarker gaps requiring regulatory acceptance exist regarding translation to humans across multiple organ systems. To address this challenge, TransBioLine (Translational Biomarker Pipeline), a consortium of EFPiA pharmaceutical companies and leading European academic institutions and bioanalytical companies, has been established to build on previous consortia work to advance clinical evaluation and regulatory qualification of new and emerging safety biomarkers addressing gaps in several major target organs: kidney, liver, pancreas, vascular system, and central nervous system. To supplement this work, unique circulating miRNA and protein signatures will be investigated. Regulatory qualification will be accomplished in collaboration with non-European consortia such as PSTC, the FNIH biomarker consortium and US DILIN. An overview of the TransBioLine project, including details on the context of use, purpose of each of the biomarker work packages and deliverables for regulatory qualification will be presented.
The host and its associated microbiome evolved as a cooperative unit that influences multi-organ host physiological and pathological outcomes. Disruption of host-microbial mutualism is associated with numerous effects upon metabolism, immune function, and organogenesis. For example, senescent periods of brain development and formation of neural circuitry are influenced by both intrinsic and extrinsic signals, including maturation of the gastrointestinal microbiome. While a connection between gut microbiota and the brain may seem unlikely, emerging data support the concept of the microbiota-gut-brain axis. Alterations in gut microbiota are associated with decreased social behavior, increased stress response, hyperactivity, reduced anxiety, and deficits in learning and memory. These findings implicate the microbiota as an omnipresent environmental factor that influences brain development, directly impacting functions of memory, behavior, personality, and higher cognition. The session contains presentations by both academic and government scientists to introduce concepts on the role of physiologic perturbations or toxic insults to the gut microbiome and its implications in neurodevelopment using both human and animal models. The first speaker illustrates how human microbial colonization at birth and life-stage transitions of the microbiome have implications in later cognitive function. A proposed mechanism for communication between gut microbes and the developing brain is presented next. Using conventional and germ-free mice, microbial signaling molecules capable of influencing host brain development and behavior have been identified. The final presentation utilizes a zebrafish model to investigate host-associated microbiota capacity to modify developmental neurotoxicity of environmental chemicals. Overall, these presentations will provide a better understanding of the function of the microbiota-gut-brain axis in neurodevelopment and the potential consequences of chemical exposures.

Complex bidirectional interactions between intestinal microbes and the nervous system may be vulnerable to perturbations via exposure to environmental chemicals, particularly during sensitive early life stages. We developed an experimental system comprised of colonized and microbe-free axenic zebrafish to test the hypothesis that microbiota modifies the developmental neurotoxicity of environmental chemicals. A light/dark locomotor activity assay was used as a functional readout of neurodevelopment. To test the effect of chemical exposures on locomotor activity and microbiota community structure, we exposed conventionally colonized zebrafish to triclosan, triclocarban, bisphenol A (BPA), BPF, or estradiol (E2) on 1, 6, 7, 8, and 9 dpf. At 10 dpf, E2 treatment had no effect on microbiota while exposure to all other test compounds perturbed community structure. We next exposed axenic, colonized and axenic zebrafish to triclosan, triclocarban, bisphenol A (BPA), BPF, or BPS and revealed that colonization status failed to modify chemical effects on locomotor activity. In comparison, E2 exposure caused hypoactivity in the light period in colonized zebrafish but had no effect on axenic zebrafish. To characterize neurobehavioral requirements for microbial colonization, we isolated strains of Actinobacter, Comamonas, and Comamonadaceae from 10 dpf colonized zebrafish. Monocolonization of axenic zebrafish with Actinobacter or Comamonas caused control-like behavior whereas monoclonization with Comamonas failed to block hyperactivity. However, conserved behavior was achieved in axenic zebrafish co-colonized with all three commensal strains. Taken together, these results suggest that recapitulation of a defined microbiota that contains sufficient diversity to reflect the full repertoire of toxicodynamic and toxicokinetic interactions between xenobiotics and microbiota may be difficult. These data also show that, while environmental chemicals often disrupt the composition of the intestinal microbiome, microbial colonization status does not generally modify the neurobehavioral effects of multiple antimicrobial and industrial chemicals. This abstract does not necessarily reflect the views of the US EPA.

The mammalian gastrointestinal (GI) tract harbors a dynamic and complex community of microorganisms, including many host physiological processes including dietary energy extraction, the production of vitamins, and protection against pathogens. Recent scientific discoveries showing an unprecedented role for the gut microbiota in host development and physiology beyond the GI tract, including the regulation of brain development and behavior has triggered a paradigm shift in our conceptualization of the origin of human brain disorders. Deciphering the molecular mechanisms underpinning the influence exerted by the gut microbiota on the developing brain has therefore become a key research priority. Recently, we demonstrated that bacterial peptidoglycans (PGN) derived from the commensal gut microbiota can be translocated into the brain and sensed by specific pattern-recognition receptors (PRRs) of the innate immune system. Using expression-profiling techniques, we demonstrated that two families of PRRs that specifically detect PGN (that is, PGN-recognition proteins and NOD-like receptors), and the PGN transporter PepT1 are highly expressed in the developing and neonatally sensitive periods of brain development and formation of neural circuitry. Therefore, these findings may have important implications in neurodevelopment using both human and animal models. The first speaker illustrates how human microbial colonization at birth and life-stage transitions of the microbiome have implications in later cognitive function. A proposed mechanism for communication between gut microbes and the developing brain is presented next. Using conventional and germ-free mice, microbial signaling molecules capable of influencing host brain development and behavior have been identified. The final presentation utilizes a zebrafish model to investigate host-associated microbiota capacity to modify developmental neurotoxicity of environmental chemicals. Overall, these presentations will provide a better understanding of the function of the microbiota-gut-brain axis in neurodevelopment and the potential consequences of chemical exposures.

The Critical Path Institute’s Predictive Safety Testing Consortium (PSTC) has been working on regulatory endorsement of translational safety biomarkers for a number of target organ toxicities. Working groups within PSTC have been established focusing on liver, kidney, skeletal muscle, vascular, pancreas, and testes, identifying and evaluating novel safety biomarkers that outperform conventional biomarkers of toxicity for these tissues. This symposium will focus on working group updates for kidney, pancreas, and vascular injury biomarkers. Each presentation will provide an overview of the progress within each working group and clinical translation efforts for the kidney injury working group, the early transition to the clinic for the vascular injury working group, and the early identification of novel pancreatic injury biomarkers in preclinical species to inform future translational work. The presentations will provide an overview of the biomarkers under evaluation, proof of concept data (both preclinical and clinical), and the status of regulatory
A significant number of drug candidates entering development present with histopathological lesions in the kidneys of animals; some at doses without projected clinical exposure margins, only in one species, or in subchronic studies after initiation of first in human (FiH) trials; and often at doses without concurrent increases in serum creatinine or blood urea nitrogen (BUN). A collection of urine biomarkers including Kim-1, clusterin, albumin, and osteopontin, among others, was qualified in 2008 for use in IND enabling rat studies to demonstrate the ability to safely monitor for drug-induced renal tubular injury, and to support their use on a case-by-case basis in clinical trials to scientifically resolve human translational relevance. To broaden this clinical qualification, a collaborative project was launched in 2011 under the FNIH consisting of pharmaceutical companies, academic investigators, CRO’s, the Critical Path Institute’s Predictive Safety Testing Consortium (PSTC) and US FDA, utilizing a learn and confirm strategy. Learning phase results will be presented for 8 biomarkers: Kim-1, clusterin, albumin, osteopontin, NGAL, cystatin-C, NAG and total protein; these urinary biomarkers were prioritized among a large set of candidates. Sufficient analytical assay performance was established for these biomarkers along with statistical thresholds of normal variability and medically meaningful thresholds. Proof-of-concept performance value was also demonstrated using existing samples from cisplatin-treated mesothelioma subjects. In addition, an algorithm was established for a composite measure of 6 biomarkers to encourage their use under a limited context of use, prior to full qualification. Confirmatory phase results will be presented which demonstrate alignment with US FDA and EMA on context of use and a statistical analysis plan. The urine biomarkers measured in this phase used samples collected from prospective trials conducted over the past 6 years in over 200 subjects from 6 sites being treated with cisplatin, tobramycin, or from non-nephrotoxic controls and the blinded data entered into a database. Both a blinded adjudication and a pre-set step-down rules-based statistical analysis were conducted in preparation for submitting data to the US FDA and EMA for a qualification decision.

Nonclinical drug-induced exocrine pancreatic injury can have a significant negative impact on the development potential of a test article. The lack of reliable, noninvasive translational biomarkers for clinical detection, monitoring or prediction of the toxicity can preclude advancing the product to clinical testing. This presentation will outline the issue from the non-clinical and clinical perspective and discuss current efforts within the pharmaceutical industry and the Critical Path Institute’s Predictive Safety Testing Consortium (PSTC) Pancreatic Injury Working Group to identify and ultimately qualify novel translational biomarkers of acinar pancreatic toxicity. The presentation will include study results of exploratory biomarkers tested in a broad range of rodent models with a variety of testing methodologies. These exploratory biomarkers include tissue-specific microRNAs, proteins and enzyme activities measured in readily accessible matrices. Tissue localization results and histologic correlations to understand the robustness of the markers’ specificity and sensitivity will also be covered. The presentation will end with an overview of the Innovative Medicines Initiative (IMI) TransBioLine pancreatic injury work package, focused on clinical evaluation of candidate microRNAs, and the synergies between the two consortia.

Developing biomarkers for vascular injury is uniquely challenging and requires creative approaches. The goals of vascular injury (VI) biomarker clinical qualification are 1) to identify a panel of safety biomarkers that can be used in conjunction with preclinical and clinical information in healthy volunteers to monitor vascular integrity in early clinical trials and 2) to indirectly demonstrate dual utility of these biomarkers as diagnostic, prognostic and efficacy biomarkers in patient populations used as surrogates for detection of drug-induced vascular injury (DIVI). The qualification approach is a panel utilizing endothelial (angiopietin 2, p-selectin, VCAM1, and thrombomodulin), smooth muscle (caldesmon, calponin-1, Smoothelin B) and inflammatory (NFκB, MMP3, IL6, IL8, IP10, GroA, TIMP1, PTX3) biomarkers to confer both sensitivity and specificity for the vascular bed. The list of biomarkers has been prioritized based on biomarker biology and nonclinical and clinical safety and pharmacodynamic data. We will advance the clinical qualification in patients with systemic vascular conditions and in patients with targeted non-infectious vascular diseases. This presentation will emphasize the need to evaluate vascular beds by ophthalmoscopy and imaging techniques such as optical coherence tomography (OCT), including angiography (OCT-A) and fluorescein angiography (FA). We will also explore bioimaging approaches for systemic vasculitides such as tracer-based methods with endothelium-depleted biomarkers, and novel MRI techniques for renal vasculitides such as dynamic susceptibility contrast (DSC), arterial spin labelling (ASL) and Intra-voxel Incoherent Motion (IVIM). To inform and supplement human patient data, biomarker performance will be assessed in animal models of retinal and systemic vascular injury. Key to this work is the development and validation of cross-species translational assays as well as the development of biostatistical approaches to enable individual and combinatorial evaluations of the biomarker panel. This presentation will provide preliminary data supporting this approach to identifying biomarkers of vascular injury.

Perfluoralkyl substances (PFAS) are a diverse group of chemicals that have been used in the production of industrial and consumer products world-wide since the 1950s. Structurally, PFAS have a characteristic hydrophobic alkylated chain that is saturated with fluorine atoms attached to a hydrophilic head. Their hydrophobic and hydrophilic properties make PFAS suitable for manufacturing of a variety of products, including nonstick cookware, liquid repellants, fire-fighting foams, protective coatings, additives, textiles, and leather and carpet goods. The two most extensively studied PFAS are perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). Although no longer manufactured in the United States, PFOA and PFOS are still used in the manufacturing of consumer products in other countries that can be imported into the country. Consequently, most people have been exposed to PFOA and PFOS unknowingly, as they tend to end up in the environment (air, water, soil, etc.). In addition, PFOA and PFOS are known to be persistent, bioaccumulative, and toxic (PBT) in animals and humans. Studies have shown that they can cause immunological, reproductive, and developmental toxicity in experimental animals, as well as liver and kidney damage. Exposure to both chemicals has also been linked to the formation of cancerous tumors in animals. There is evidence that humans exposed to PFOA and PFOS can develop increased cholesterol levels, thyroid hormone disruption, and cancer.

The goal of this session is to bring together graduate students and postdoctoral scientists from different sectors to address the emerging scientific, environmental health, and regulatory issues raised by PFOA and PFOS. The first speaker will present on the use of various zebrafish assays to assess development and neurotoxicity of PFOA and PFOS. The second speaker will discuss the potential mechanisms by which PFOA and PFOS cause fetal growth restriction in mice. Finally, the last speaker will talk about the modulatory effects of PFOA and PFOS on Gabaa receptor function and neuronal network activity in primary rat cortical neurons.

Perfluoralkyl substances (PFAS) are structurally diverse class of industrial chemicals with widespread environmental occurrence. Exposure to long-chain PFAS like perfluorooctane sulfonic acid (PFOS) or perfluorooctanoic acid (PFOA) are associated with developmental toxicity and neurotoxicity. Long-chain PFAS have therefore been replaced with short-chain and polyfluorooether compounds. For hazard identification of replacement PFAS, developmental toxicity (DevTox) and developmental neurotoxicity (DNT) using locomotor activity as functional readout were assessed in 6 day post-fertilization (6 dpf) zebrafish exposed to 0.044-80.0 μM ammonium 4,8-di-oxa-3H-perfluoronoanoate (ADONA), GenX (HFPO-DA), Nafion byproduct 1 (Nafion BP1), perfluorohexanoic acid (PFHxa), PFOA, PFOS, potassium perfluorohexane-1-sulfonate (PFHxs), or 0.4% DMSO with daily renewal on 0-5 dpf.
Exposure to 3.1-27.2 μM PFOS or 80 μM PFHxS resulted in failed swim bladder inflation and abnormal ventroflexion of the tail. ADONA, GenX, PFHxA, PFOA, or Nafion BP1 exposures were negative for DevTox. The DNT assay consisted of two consecutive 10 min light periods (L1 and L2) and two consecutive 10 min dark periods (D1 and D2). Exposure to 0.1-3.1 μM PFOS or 4.4-44.8 μM PFHxS triggered locomotor hyperactivity in the L1, L2, and D1 periods while exposure to 14.0-25.1 μM PFHxA produced hyperactivity in the D1 and D2 phases. All other test chemicals were negative. Because exposure to structurally similar sulfonic acid-containing PFAS (i.e. PFOS and PFHxS) caused identical morphological and behavioral phenotypes, we hypothesized that this group of PFAS has a shared toxicity mechanism. To address this, perfluorooctanesulfonic acid (PFOS, four-carbon), perfluorodecanesulfonic acid (PFHxS, seven-carbon), and PFOA (eight-carbon) were assessed for DevTox and DNT. Chemical potency for DevTox and hyperactivity in the DNT assay was correlated with carbon chain length (PFOS>PFHxS>PFOA; PFBS was negative). Together, exposure to PFOS, PFHxS, or PFHxA caused DevTox while exposure to non-teratogenic concentrations of PFOS, PFHxS, or PFHxA provoked hyperactivity. Taken together, this work identified relationships between chemical structures and in vivo phenotypes that may arise from putative shared mechanisms of PFAS toxicity. This abstract does not necessarily reflect US EPA policy.

2536 An In Vitro Screen of a Panel of Perfluoroalkyl Substances and an In Vivo Assessment of Effects on Placental and Fetal Growth

B. Blake. University of North Carolina at Chapel Hill, Chapel Hill, NC.

Perfluoroalkyl substances (PFASs) are ubiquitous environmental contaminants and are associated with fetal growth restriction (FGR). Perfluorooctanoic acid (PFOA) is one of the most well-studied PFASs and it is estimated that a 1 ng/mL increase in serum PFOA is associated with an 18.9g decrease in human birth weight. The mechanism through which PFOA affects fetal growth is not known and we hypothesize that the feto-placental unit is a target. PFOA is one of thousands of PFASs, and determining modes of action against the developing fetus as well as a method for PFAS prioritization are necessary. A high-throughput, multiplexed screen was developed using human placental Jeg-3 cells for PFAS prioritization. The 24h screen included assays to determine the effects of PFAS ranging from 50-500μM on proliferation, cell viability, and mitochondrial function. Doses were selected based off assay optimization due to toxicological implications. In-vivo-relevant doses for chemicals for which human exposures have yet to be defined. Of 33 PFAS screened, 56% showed effects on proliferation, 36% on mitochondrial function, and 39% on cell viability. To determine a potential mechanism of FGR for PFOA and evaluate whether its replacement, GenX, has similar effects on placental and fetal growth, pregnant CD-1 mice were exposed to PFOA (1 and 5 mg/kg/day) or GenX (2 and 10 mg/kg/day) from embryonic day 1.5 until E11.5 or E17.5 (n=5-8 per group). Doses were selected based on previous studies in our lab. Maternal weight gain was elevated at E11.5 in a dose-response manner with effects most prominent in mice exposed to GenX at 10 mg/kg/day (21% increased weight) followed by PFOA at 5 mg/kg/day (10% increased weight). At E17.5, mice exposed to 5 mg/kg/day PFOA or 10 mg/kg/day GenX had increased placental weights (18% and 22% respectively), decreased fetal weights (21% and 5% respectively), decreased fetal length (6% and 0.6% respectively) and decreased fetus/placenta ratios (32% and 21% respectively). These data suggest both PFOA and GenX can affect the placenta and embryo, possibly via diverging mechanisms where PFOA appears to affect both the fetus and placenta, whereas GenX appears to primarily impact the placenta. These findings implicate GenX as a potential public health threat rather than a safe alternative.

2537 Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoate (PFOA) Differentially Modulate GABAA Receptor Function and Spontaneous Neuronal Network Activity

A. M. Tukker, Utrecht University, Utrecht, Netherlands.

There is increasing concern about the possible (developmental) neurotoxic effects of environmental pollutants, including the highly persistent compounds perfluorooctanoic acid (PFOA) and perfluorooctanoic acid (PFOA). Earlier studies indicated that the inhibitory GABAA receptor is an important regulator in neuronal development and function, but also a sensitive target for persistent organic pollutants. Therefore, this study aims to explore the effect of two perfluorinated compounds (PFCs) on GABAA receptor function and neuronal network activity. Using two-electrode voltage-clamp, we demonstrate that PFOS (0.001-100 μM) and PFOA (0.001-100 μM) induced concentration-dependent antagonistic effects on human α1β2γ2L GABAA receptors, expressed in Xenopus laevis oocytes. Notably, the inhibitory effects of PFOS and PFOA were already evident at human-relevant submicromolar concentrations (0.1 μM). While the inhibition by PFOA was independent of the GABA concentration (LOEC 1 μM in co-application with 1 mM GABA), the degree of inhibition evoked by PFOS increased at higher GABA concentrations (LOEC 0.1 μM in co-application with 1 mM GABA). As PFC-induced inhibition of GABAA receptors is hypothesized to increase neuronal activity, we assessed the effects of PFOS and PFOA on spontaneous neuronal network activity in primary rat cortical cultures grown on multi-well micro-electrode arrays (mwMEA). Surprisingly, only the highest concentration tested (100μM) induced a transient (PFOA) or persistent (PFOS) inhibition of neuronal activity, whereas lower concentrations were without effect. These data therefore indicate that the variability in PFCs’ structurally similar sulfonic-acid containing PFAS (i.e. PFOS and PFHxS) caused identiﬁcation of modes of action against the developing fetus as well as a method for PFAS prioritization are necessary. A high-throughput, multiplexed screen was developed using human placental Jeg-3 cells for PFAS prioritization. The 24h screen included assays to determine the effects of PFAS ranging from 50-500μM on proliferation, cell viability, and mitochondrial function. Doses were selected based off assay optimization due to toxicological implications. In-vivo-relevant doses for chemicals for which human exposures have yet to be defined. Of 33 PFAS screened, 56% showed effects on proliferation, 36% on mitochondrial function, and 39% on cell viability. To determine a potential mechanism of FGR for PFOA and evaluate whether its replacement, GenX, has similar effects on placental and fetal growth, pregnant CD-1 mice were exposed to PFOA (1 and 5 mg/kg/day) or GenX (2 and 10 mg/kg/day) from embryonic day 1.5 until E11.5 or E17.5 (n=5-8 per group). Doses were selected based on previous studies in our lab. Maternal weight gain was elevated at E11.5 in a dose-response manner with effects most prominent in mice exposed to GenX at 10 mg/kg/day (21% increased weight) followed by PFOA at 5 mg/kg/day (10% increased weight). At E17.5, mice exposed to 5 mg/kg/day PFOA or 10 mg/kg/day GenX had increased placental weights (18% and 22% respectively), decreased fetal weights (21% and 5% respectively), decreased fetal length (6% and 0.6% respectively) and decreased fetus/placenta ratios (32% and 21% respectively). These data suggest both PFOA and GenX can affect the placenta and embryo, possibly via diverging mechanisms where PFOA appears to affect both the fetus and placenta, whereas GenX appears to primarily impact the placenta. These findings implicate GenX as a potential public health threat rather than a safe alternative.

2538 Species Relevance: Approaches to Determine the Most Relevant Species for Safety Assessment of Pharmaceutical Products

M. Delatte. US FDA, Silver Spring, MD.

Most development programs for pharmaceutical products employ a rodent and nonrodent species for assessment of potential hazards. In a risk assessment, findings from the most relevant species are typically used to determine safe clinical exposure levels. Many scientists typically conclude that the most sensitive species is the most relevant species. However, there are instances in which the most relevant species may be selected based on anatomy, physiology, target site for efficacy, pharmacokinetic profile, or other relevant factors. Identifying the most relevant species is a critical step in hazard/risk assessment given the need to determine that the hazards identified are clinically relevant and representative of effects likely to occur in humans, based on qualitative and quantitative aspects of the hazard(s) identified. This session will introduce the attendees to multiple factors that may impact species relevance when conducting a hazard assessment. The second talk will discuss risk assessment of small molecules and approaches used to determine the most relevant species in pharmacology and general toxicology studies. The third talk will discuss approaches used to determine the most relevant rodent species for carcinogenicity assessment of small molecules. The fourth presentation will discuss hazard assessment of biologic products and approaches used to determine the most relevant species for reproductive toxicology studies and carcinogenicity assessment. Together, the information presented in these talks will highlight the importance of and provide a framework for selecting the most relevant species when conducting a safety assessment.

2539 Comparative Anatomy and Physiology in Animal Species Commonly Used for Drug Safety Testing

J. Turk, Amgen, St. Louis, MO. Sponsor: M. Delatte.

Toxicology studies involve the use of a wide range of laboratory animals that present unique anatomical and physiological characteristics relevant to species selection for toxicology assessments. This introductory session will provide an overview of salient species differences, but also similarities from rodents to non-rodents, and will ultimately address translational considerations as the comparison is extended to humans. The homology across species differs with organ systems which has interpretation implications when evaluating drug-induced adverse effects. Organ system differences mandate a scientific review of anatomical and physiological features during species selection. Even when present, anatomical differences may not significantly impact tissue response to drug exposure and consequently may have minimal implications relative to species relevance in the context of toxicology studies. As the session introduces comparative anatomy and physiology across species, it will set the stage for an in-depth discussion on factors involved in the species selection process for drug safety testing.
Non-clinical toxicology and pharmacology studies involve risk identification but also risk characterization. While the value of different animal species may be equivalent for risk identification, differences may emerge during risk characterization and establishment of safety margins. In addition, the value of the various animal species used for risk identification and characterization varies based on the liability and types of drug induced adverse effects that are evaluated. As such, this session will explore divergences and similarities observed in animal species with implications for interpretation in toxicology and pharmacology. It will discuss well known species characteristics but also unique cases involving vehicle reactions, species specific metabolism, or clinically irrelevant animal features, all in the context of a science-driven approach.

Lastly, translational considerations will be discussed related to strategic decisions when contemplating selection of the most sensitive species compared to the most relevant species.

Species Selection in Toxicology and Pharmacology Studies: Challenges, Opportunities, and Lessons Learned

S. Authier. Citoxlab North America, Laval, QC, Canada. Sponsor: M. Delatte

An overview of current models, including details regarding the engraftment of specific immune cell subsets, and their potential application. The limitations of each of these models also will be discussed. Examples of current applications will be discussed, including assessment of their value in evaluating cytokine release, graft-versus-host disease, and comparative assessments of biosimilar products. Recent advances in humanized mouse models will be presented including novel, rapid, and sensitive in vivo models to assess individual responses to immune therapy agents and in combination therapies.

Species Considerations for Nonclinical Carcinogenicity Evaluations

O. McMaster. US FDA, Silver Spring, MD.

Carcinogenicity studies are conducted for drug products if the expected clinical use is for = six months and/or if there is cause for concern, such as preneoplastic lesions. The conventional approach typically includes one long-term rodent carcinogenicity study, plus one other study (such as a short- or medium-term in vivo rodent test system such as TgRasH2 mouse or a long-term rodent carcinogenicity study in a second rodent species). The choice of species for a long-term carcinogenicity study should be based on consideration of the drug pharmacology, toxicology, toxicokinetics and route of administration. The conventional approach may not be appropriate for biopharmaceuticals due to the unique and diverse structural and biological properties including species specificity. Selecting relevant animal species for testing should be done very meticulously as studies in non-relevant species could be misleading and are discouraged. Even when the results of a carcinogenicity study are unequivocally positive, the data may be irrelevant for assessing risk to humans based on the species in which the finding was detected. This talk will discuss the various factors which must be considered in selecting the species/animal models that best predict the risk of human carcinogenicity.

Biologic Product Species Selection: When Data Conflict

J. Dubinion. US FDA, Silver Spring, MD. Sponsor: M. Delatte

ICH Guidance S6 (R1) provides assay recommendations (sequence homology, target binding affinity, receptor/ligand occupancy, and functional activity) for determining species relevancy for biologic drug products. While the guidance suggests that tissue cross reactivity studies are of limited value for species selection, it also describes specific cases where comparison of tissue binding profiles between human and animal tissues can guide selection of toxicology species. This flexibility can sometimes be misinterpreted by sponsors especially when the data from the recommended assays conflict with findings from tissue cross reactivity studies. This talk will discuss hazard assessment of biologic drug products in relation to species selection. It will highlight the importance of selecting relevant species based on the best scientific data early in drug development, and methods to prevent costly changes in animal species for completion of reproductive developmental toxicity/carcinogenicity later in product development.

The Current Application, Limitations, and Recent Advances in Humanized Mouse Models for Evaluations of Immune Function and Preclinical Immunotoxicology Studies

M. Collinge. Pfizer, Inc., Groton, CT.

Significant advances have been made in recent years in the development of humanized mice for use in preclinical pharmacology and toxicology studies to support the development of pharmaceutical biotherapeutics. Multiple models currently exist, and the selection of the appropriate model is critical to provide meaningful and clinically translatable data. This session will provide

Species Selection in Toxicology and Pharmacology Studies: Challenges, Opportunities, and Lessons Learned

S. Authier. Citoxlab North America, Laval, QC, Canada. Sponsor: M. Delatte

NSG Mice Deficient in Murine MHC Class I and Class II Expression Support Engraftment of Functional Human T Cells in the Absence of Acute Xenogeneic GVHD following Injection of PBMC

M. Brehm. University of Massachusetts Medical School, Worcester, MA. Sponsor: M. Collinge

Recent Advances in Humanized Mouse Models for Toxicology Assessment of Novel Therapeutics

J. Keck. Jackson Laboratory, Sacramento, CA. Sponsor: M. Collinge

Immunotherapy is becoming a powerful therapeutic method in cancer and autoimmunology diseases. Monoclonal antibodies (mAbs) have been successfully used for immunotherapy for many years. Many of these mAbs are targeted against proteins on the surface of immune cells, especially T-cells and B-cells. However, mAbs can have a variety of adverse effects at the time of infusion, such as cytokine release syndrome (CRS), which can be lethal. There are generally two existing methods for immunotoxicity testing prior to clinical trials of a drug: namely, in vivo testing in animal models and in vitro human peripheral blood mononuclear cell (PBMC) assays. Unfortunately, these two methods
developed a tiered approach to assess the safety of botanicals which includes an assessment of botanical-drug interaction (BDI). The scientific literature is replete with reports of various botanical extracts and/or constituents as potent inhibitors of drug metabolizing enzymes and transporters. Unfortunately, most of these studies use simplistic in vitro screening-based systems without follow-up in more physiologically-relevant models. Additionally, the potential for botanical extracts to induce metabolism enzymes and/or transporters is rarely studied; particularly in in vitro systems. Thus, in the rare instances when clinical studies are conducted to confirm botanical-drug interaction (BDI) potential, there is usually a poor correlation between in vitro studies and clinically-relevant changes in the pharmacokinetics of drugs under study. Recently, our laboratory has proposed a new paradigm to assess BDIs using sandwich-cultured human hepatocytes (SCHH) and an in vitro clearance approach that treats the complex botanical mixture as a single entity, regardless of the constituent profile. Our experimental design captures major clinically-relevant hepatic pathways and data output that makes direct predictions of clinical BDI and the impact on a victim drug (e.g. liver clearance). In studying the complex mixture in this way, we can capture the overall net effect of any synergism or additive effects that might be occurring.

**S 2547 When “Natural” Is Not Synonymous with “Safe”: Toxicity of Natural Products Alone and in Combination with Pharmaceutical Agents**

D. Mendrick, US FDA, Silver Spring, MD.

Retail sales of vitamins and nutritional supplements have increased from $23.8 billion in 2007 to $36.1 billion in 2017, with more than 70% of Americans currently taking some dietary supplement daily. While many equate these “natural” products with “safe,” it is well recognized by scientists that active ingredients in natural products can result in toxicity. Additionally, when these products are used in concert with pharmaceutical agents, they can alter drug metabolism and drug delivery, thereby enhancing or reducing the therapeutic effect of the drug(s). However, there is no current requirement for dietary supplements to be registered with the US FDA, and only post-marketing adverse events have to be reported. With the widespread use of nutritional supplements, these dietary supplement-drug interactions can be underappreciated and cause the failure of promising drugs. This symposium will explore the toxicity of natural products alone and in combination with conventional drugs. Systematic approaches for assessing natural product-drug interactions, including in vitro, PBPK modeling, and human clinical studies, will be presented. Methodology to handle the complex mixtures represented by natural products in various test systems also will be discussed. Finally, a case study involving a natural product (cannabidiol) and potential for drug interaction will be highlighted.

**S 2548 Causative Ingredients and Mechanisms Underlying Interactions between Prescription and Nonprescription Medications and Natural Products**

M. F. Paine. Washington State University, Spokane, WA. Sponsor: D. Mendrick

Common mechanisms underlying pharmacokinetic interactions between natural products (NPs) and conventional drugs include induction and inhibition of drug metabolizing enzymes and transporters, leading to altered systemic or tissue drug concentrations and potentially, suboptimal therapeutic effects. Rigorous guidelines for assessing the risk of NP-drug interactions are non-existent, due in part to NPs being inherently complex mixtures that vary substantially in bioactive ingredient composition. The National Center for Complementary and Integrative Health established the Center of Excellence for Natural Product-Drug Interaction (NaPDI) Research in September, 2015. A key deliverable of the NaPDI Center is a set of recommended approaches to guide researchers in the proper conduct of NP-drug interaction studies. These approaches will be based on results generated from a series of ongoing interaction projects focused on four systematically selected NPs as precipitants of NP-drug interaction effects. The data generated from these projects are being entered into a data repository that will be disseminated to researchers via a public access portal. The efforts of the NaPDI Center should lead to improved design of future NP-drug interaction research and eventually, improved decisions about the optimal management of these complex interactions.

**S 2549 Botanical-Drug Interaction Assessment: A Critical Component of a Systematic Approach to Botanical Safety**

A. Roe. Procter & Gamble Company, Cincinnati, OH.

Exposure to botanicals in dietary supplements is increasing across many geographic areas; with increased expectations from consumers, regulators, and industry stewards centered on quality and safety of these products. We have observed variability in artisanal CBD products and the limitations in extrapolating the known information on pharmacology of purified CBD to artisanal products will be discussed.

**S 2550 Cannabidiol Pharmacology and Drug-Drug Interactions with Anti-Epileptic Drugs (AEDs)**


There has been increasing interest in the use of cannabinoids in the treatment of epilepsy. While there is indication that some cannabinoids, particularly cannabidiol (CBD), are effective in treating epilepsy, there is an assumption frequently observed in the community that CBD is completely safe and without side effects or drug-drug interactions since it is plant-based. A highly purified CBD solution has recently been US FDA approved. This presentation will introduce the pharmacology of cannabidiol, including proposed mechanisms of action, pharmacokinetics, metabolism, and excretion. Further, CBD’s drug-drug interactions with other anti-epileptic drugs (AEDs) will be discussed, as documented, clinically significant interactions with clobazam and valproate have been identified and are likely due to CBD’s effects on certain CYP450 enzymes. Other potential pharmacokinetic interactions have been seen with topiramate, zonisamide, rufinamide, and eslicarbazepine. However, more data are needed to confirm many of these potential drug-drug interactions. In closing, variability in artisanal CBD products and the limitations in extrapolating the known information on pharmacology of purified CBD to artisanal products will be discussed.

**W 2551 New Approaches Using Mode-of-Action to Predict Acute and Systemic Toxicity**

C. Willett. Humane Society of the United States, Washington, DC.

There is a transformation occurring in toxicology: a shift toward characterizing chemical safety based on an understanding of the biomolecular activity of the chemical coupled with a deeper understanding of how that activity can lead to adverse effects at the organ, individual, or population level. New approach methodologies (NAMs) can be used to identify a chemical’s mode of action (MoA), including the molecular initiating events (MIEs) and downstream key events possibly leading to adverse outcomes (AOs). Application of this NaPDI paradigm is furthest advanced for biology where the MIEs are better established, such as skin sensitization, genetic toxicity, and endocrine models. However, for systemic acute or repeat-dose toxicity, where several possible MIEs may be involved, the ability to identify specific MIEs and use in vitro data to predict complex toxicological outcomes is much less mature and urgently needed. This session provides three innovative approaches to predicting chemical safety by identification of MoA based on NAM data: one for acute mammalian systemic toxicity, and two different approaches for repeat-dose target organ toxicity. The presentations will provide a synopsis of the state-of-the-science regarding availability of databases for these endpoints, as well as application of integrating computational and in vitro models in a predictive fashion. The session will emphasize successes and limitations in current NAMs for these endpoints and highlight the priority needs for future dedicated research to improve modeling. Discussion will focus on what is needed to bring these approaches into broad regulatory use.
We have developed in silico mechanistic profilers that identify putative molecular initiating events. We built these either using defined SMARTS codes or by using machine learning and artificial intelligence with structural scaffolding and fingerprinting of in vitro data, which is integrated in some cases with a consensus model using protein docking binding energies. Uniquely, our models can provide both positive and negative predictivity when there are enough specific negative in vitro data. We are aligning the predicted in silico targets with appropriate in vitro models in an integrated manner to predict in vivo toxicity. As proof of concept, we will show application of this integrated approach to acute mammalian toxicity wherein we have profilers that account for most GHS 1-3 MIEs. We also show both the merits and limitations of down-stream in vitro models for basal cytotoxicity. For instance, we demonstrate using in silico approaches to identify in vitro models that lack comprehensive predictivity due to poor metabolic capability, inability to delineate cytotoxicity from known cytotoxic reactive chemistries, or by questionable in vivo data curation results. These studies will highlight potential research gaps and future projects.

Toxic effects following repeated dose exposure are one of the key drivers for assigning regulation classifications to chemicals. Determining whether a substance has a specific mode of action, which may drive toxicity in one or more organs, or is non-specific in its action is an essential part of determining toxic potency, and hence will influence regulatory classifications. Data from New Approach Methodologies (NAMs) can assist in identifying modes of action associated with regulatory classifications, although no overarching strategy is yet available. To resolve this, a study was initiated to investigate repeated dose toxicity data-associated specific target organ toxicity - repeat exposure (STOT-RE) classifications for substances for which in silico profiling was undertaken and ToxCast data were available. Data from NAMs supported grouping of known specific mechanisms (e.g. for hepatotoxicity) which could be associated with classifications. It was more difficult to develop definitive associations between NAMs and non-specific mechanisms of action, although a combination of the absence of in silico alerts and ToxCast hits was indicative of substances with low toxicity. The funding of CEFIC LRI Project AIMT B is gratefully acknowledged.

Although predicting adverse effects has traditionally concentrated on observations at the organ and organismal level, we know that these adverse events are the culmination of a chain of events that begins with an interaction between the exogenous chemical and an endogenous target at the molecular level. Accordingly, we have concentrated on trying to understand the modes of action of toxicants at a molecular level, both by analyzing chemical features and by identifying the initial biological responses to toxicants. We have curated large in vivo data sets of rodent and rabbit developmental and reproductive toxicity (DART) data as well as human data, to catalog the kinds of chemical structures that are associated with DART. Using these data, we are deriving mode of action ontologies that link molecular targets to in vivo effects. We are supporting our assumptions about mode of action by using global gene expression analysis in a series of cell types that provide biological diversity. This talk will demonstrate progress on the mode of action ontology, and provide examples of how the ontology and data from toxicogenomics provide support for prediction of toxicity by read across. In the process, we will gain greater understanding of the universe of modes of action for DART, which should ultimately be robust enough to support predictions even for chemicals that have no analogs that have in vivo data.

Echocardiography provides a non-invasive means to assess cardiac structure and function and is widely used clinically to assess left ventricular function, hypertrophy, valvular disease, and myocardial infarction. Increasingly, echocardiography has become a sought-after tool in nonclinical research to make informed decisions on intended pharmacology and/or off-target actions of test articles under development. This session will aim to provide an overview of the utility of echocardiography in nonclinical research, including the latest technologies, species and study design considerations, and a regulatory viewpoint on the utility of nonclinical echocardiography data in the assessment of Investigational New Drug (IND) submissions and subsequent clinical trials. The session will start with an outline of the use of small (rodents) and large (dogs, monkeys) animal echocardiography, including a description of common endpoints assessed. In addition, the first presenter will address the utility of echocardiography in healthy animals and animal models of cardiac disease as part of nonclinical safety assessment. The second presentation will address a range of anatomic, behavioral, and hemodynamic factors that affect quantitative analyses, including common design elements used to maximize data quality and effect size detection thresholds, and potential pitfalls to avoid. The third talk will discuss how nonclinical ultrasound data generated in animals are utilized and interpreted as part of an IND submission and how these data may impact early clinical trials. The target audience is toxicologists who may have limited exposure to the utility of echocardiography in nonclinical animal studies and are looking to expand their knowledge in the area. By the end of the session, attendees should better understand the technical considerations and strategies for employing echocardiography in nonclinical animal studies and how animal-based echocardiography data generated as part of a drug safety assessment program may impact IND submission and subsequent clinical trials. Based on the fact that cardiovascular liabilities continue to be a leading cause of drug attrition in late-stage clinical trials and post-market approval, it is expected that additional measures to assess cardiac function will be of great interest to the toxicology and drug development communities.

Echocardiography provides a non-invasive means to assess cardiac structure and function. High-frequency ultrasound with resolution to 30 µm and frame rates of over 30 Hz can capture the minute detail of rapidly moving rodent hearts. Clinical ultrasound platforms are perfectly suited to imaging larger animals. Therefore, both systems are ideal for nonclinical imaging and drug screening in a wide range of animal species. The utility of echocardiography including measures of wall thickness, chamber dimensions, valve structure, and various wall thickness in 2, 3, and even 4-dimensions will be presented. Echocardiography can also assess cardiac performance including measures of systolic and diastolic function and tissue movement via strain. Doppler measures of blood velocities moving through valves and other blood vessels will add additional insight. This presentation will cover the range of measurements used in cardiac ultrasound in healthy animals and animal models of cardiac disease that may be employed as part of nonclinical safety assessment studies.

Echocardiography is increasingly used as an assessment of the cardiac effects of test articles. The modality permits non-invasive, repetitive, rapid assessments of cardiac size, including markers for myocardial mass, indices of hemodynamics, and quantitative indices for systolic and diastolic function. Anatomic, behavioral, and hemodynamic factors that affect quantitative analysis will be described including common design elements used to maximize data quality and effect size detection thresholds, and potential pitfalls to avoid will be discussed. Interpretation schemes will be discussed, including principles such as asymmetric cause/effect analysis. Hypothetical examples of optimum versus non-optimum use of echocardiography in test article development will be presented.
The US FDA supports efforts to improve the informative and translational value of nonclinical studies. These efforts are in alignment with the trends of increasing, reducing, and replacing animal studies while also increasing the safety of clinical trials for participants. Recent advances in echocardiography give this imaging modality the potential to have a significant impact on nonclinical safety assessment. Echocardiography is a non-invasive or minimally invasive technique that offers in-life visualization of several components of cardiac function and blood flow and quantitative assessment of physiologic or toxicologic changes. This real-time evaluation of cardiac function offers several possible roles in nonclinical safety assessment, with the potential for translation to the clinic. Its successful implementation in nonclinical studies and submission to regulatory agencies is dependent on several key factors, including the observer, analysis, and reporting. This presentation will discuss the potential use of echocardiography in nonclinical studies and the types of beneficial information to include when submitting to regulatory agencies.

Tips for Improving Scientific Communication with a General Audience

J. Shannahah, Purdue University, West Lafayette, IN.

For research to broadly and positively impact public health, it must be efficiently communicated to, and understood by, the general public. The majority of university-level scientific training focuses on performing cutting-edge research and sharing those findings with other scientists within one’s own field. In a time when information is readily accessible, ensuring effective and accurate scientific messaging through community outreach is necessary for maximizing societal impact and understanding. This is true during one-on-one conversations with nonscientists, and through interactions utilizing social and mass media. Deficiencies in the capacity to share science-related topics with media and the general public result in misinterpretation of conclusions and decreased community engagement in science. This session is designed to bring in scientific outreach experts to share tips and strategies for researchers to successfully communicate science with the general public. Speakers will focus on (1) individual interactions, (2) controlling your message, (3) the use of innovative social media platforms, and (4) effective utilization of mass media. These interactive presentations will include real-world examples of successful scientific communication as well as illustrations of common errors scientists are prone to committing. These discussions will be highly applicable to all attendees, including graduate students, postdoctoral trainees, and senior toxicologists. This session will allow both trainees and seasoned toxicologists to learn and implement this increasingly useful and necessary skill set.

Establishing Effective Alternatives for Acute Oral and Inhalation Systemic Toxicity Testing

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Acute systemic toxicity testing is required by regulatory agencies worldwide, providing the basis for hazard labeling and risk management of industrial chemicals, agrochemical formulations, and pharmaceuticals, and represents the highest cumulative animal use across chemical sectors. The development of test methods that reduce or replace animal use for acute systemic toxicity tests is one of the highest priority activities of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), which is comprised of representatives from 16 US federal regulatory and research agencies that use or generate toxicological testing information. Despite the multitude of in vitro and in silico methods, a lack of regulatory acceptance of defined approaches prevents the widespread adoption of these approaches by industry. This session will demonstrate how engaging regulators and stakeholders up-front facilitates effective integration of alternatives, ensuring a path to success in reducing the use of animals in acute testing. Efforts in the United States to identify, develop, validate, and implement alternatives to the traditional acute systemic toxicity tests associated with oral and inhalation exposures will be highlighted. Overall, this session will review the current status of developing alternative approaches to acute systemic toxicity testing to meet agency needs, challenges in integrating new methods, and approaches to facilitate the adoption of these alternative methods in the near term and achieve the goal of significantly reducing animal use in acute toxicity testing by 2020.

Derivation of a Dataset for Modeling Acute Oral Toxicity and Variability Assessment of In Vivo LD50 Data

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Alternative models developed for estimating acute systemic toxicity are generally evaluated using in vivo LD50 values. However, in vivo acute systemic toxicity studies can produce variable results, even when conducted according to accepted test guidelines. This variability can make assessment of alternative models extremely challenging. To characterize the variability of in vivo acute systemic toxicity data, we examined a large compilation of LD50 values reported in rat oral 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 21,210 rat oral LD50 values representing 15,698 unique chemicals. A subset of >1000 chemicals that had been evaluated in at least three independent rat oral acute toxicity studies were used to assess variability. Chemicals with the highest variability were manually curated to ensure data reliability. Of this subset, many chemicals were identified that had LD50 values ranging across at least two, and sometimes three, orders of magnitude. This degree of variability can confound hazard categorization, particularly when LD50 values fell into multiple different Global Harmonization Scheme (GHS) oral acute toxicity labeling categories or US EPA hazard categories. These findings underscore the importance of considering an appropriate margin of uncertainty when using 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 alternative models.
ultimately produce publicly accessible CaT莫斯 predictions via the US EPA’s CompTox Dashboard interface and the consensus models will be available for further chemical screening via the OPERA predictive tool.

2564 In Silico and In Vitro Approaches to Assess Inhalation Toxicity Testing
A. Clippinger, PETA International Science Consortium Ltd., Norfolk, VA.

Scientific and ethical drivers have led to interest in developing human-relevant, mechanistically based approaches for inhalation toxicity testing that can be used instead of animals. As a result, multi-stakeholder collaborations have formed between industry, government, non-profit organizations, method developers, and academia to advance these approaches. This presentation will highlight progress and ongoing work of an international multi-stakeholder collaborative that has focused on determining federal agency needs, curating existing data, and identifying gaps in in silico or in vitro methods available to assess toxicity following inhalation exposures. Partnerships have been established to better understand inhalation dosimetry of various agents and mechanisms of toxicity to support the development of adverse outcome pathways (AOPs). AOPs can be used to direct the design of integrated approaches to testing and assessment that evaluate all existing data, and incorporate dosimetry considerations, physicochemical property information, and in vitro and computational approaches in an integrative fashion. Proof-of-concept testing is being conducted to show the utility of non-animal approaches to predict the toxicity of inhaled substances. The ideal testing approach will vary depending on the test substance and purpose of the study, and results from proof-of-concept testing will be used to further the development and optimization of testing approaches that will be fit for regulatory decision-making.

2565 Integrating Nonanimal Alternative Approaches to Assess the Risk to Human Health from Inhaled Materials
J. Hotchkiss, Dow Chemical Company, Midland, MI.

There is need to identify nonanimal alternatives to assess the acute toxicity of inhaled materials for hazard identification. In vitro exposure of 3D human organotypic epithelial cultures grown at the air liquid interface (ALI) may provide an immediate path to assess airway epithelial responses to deposited and adsorbed aerosols, vapors and gases that are relevant for human risk assessment. Commercially available organotypic airway cultures reproduce many features of in vivo human respiratory epithelium, including 3D epithelial structure, functional cilia, mucus secretion, barrier properties and metabolic activity. This study examined cytotoxicity, inflammatory, metaplastic and oxidant injury exposure response profiles of large (Epithelix Mucilair HF™; MAF) and small (Epithelix Smallair HF; SAF) airway epithelial cultures. Test materials (SDS; direct cytotoxicity, TNF-α; induction of acute inflammation response, IL-13; induction of mucous cell metaplasia, H2O2; oxidant injury) with well-defined airway epithelial responses in vivo and in vitro were applied directly to the apical epithelial surface via pipetting or as a liquid aerosol using a VitroCell Cloud in vitro exposure system. The results of this study underscore the need to measure multiple endpoints to evaluate the acute exposure response profiles and the ability of the large and small airway epithelial cultures to recover/adapt to injury. These data also confirm the need to use deposited/absorbed dose and not exposure atmosphere concentration when assessing the acute toxicity of inhaled test materials using alternative in vitro test systems. This in vitro model is a key component to develop an integrated approach for acute inhalation toxicity assessment that will integrate cheminformatics evaluation of the potential mode of action based on the molecular initiating event (MIE) and key events identified by the appropriate adverse outcome pathway (AOP).

2566 Progress toward Charting the Course for Improving Carcinogenicity Assessments of Human Pharmaceuticals and Pesticides
J. Van der Laan, Medicines Evaluation Board, Utrecht, Netherlands.

Health authorities responsible for regulating pharmaceuticals and pesticides request studies to determine carcinogenic potential. These studies conform to Guidelines of the International Council on Harmonization (ICH S1) for drugs and OECD guidelines for pesticides. For drugs these studies are requested to be conducted when human treatment is necessary for longer than six months; for crop protection chemicals they are required for most exposure scenarios. The value of the rodent bioassays continues to be questioned because of their lack of relevance to humans. Retrospective analyses of various datasets (PhRMA, US FDA, JPMa, and EU) concluded that the outcome of these rodent studies could be predicted as follows: negative predictions can be made when carcinogenic signals such as the absence of hyperplasia in a six-months study and when certain pharmacological properties are absent, whereas positive predictions are possible when such signals are present. In-between compounds remain for which the outcome is equivocal and where experimental studies may add value to identify real hazards. These hypotheses stimulated ICH to evaluate compounds in a prospective way in an ongoing exercise. US EPA and the pesticide industry are similarly stimulated to consider harmonization on such alternative approaches. This symposium is intended to provide transparency into the progress being made to establish internationally harmonized approaches to enable more flexible carcinogenicity assessment strategies focused on mechanisms while reducing reliance on the two-year rodent bioassay. Case examples for pharmaceutical and pesticide development will be provided that demonstrate how successful implementation might look. The opportunities that emerging new technologies and rich scientific information sources can play to impact the future evolution of this flexible framework will be described.

2567 ICH S1 Project Status Update: The Need for Carcinogenicity assessment
J. W. van der Laan, Medicines Evaluation Board, Utrecht, Netherlands.

Sponsors are encouraged to submit Carcinogenicity Assessment Documents (CADs) to Drug Regulatory Authorities (DRA’s) for all investigational pharmaceuticals with ongoing or planned 2-yr rat carcinogenicity studies. The CAD addresses a weight of evidence approach of the carcinogenic risk of an investigational drug. This approach is based on available pharmacology and toxicology data and a rationale for why the conduct of long-term studies would not add value to that assessment. Drug Regulatory Authorities independently review the submitted documents and evaluate the degree of concordance with Sponsors. During this prospective evaluation period waiver requests will not be granted, as the data are collected solely for real world experience. Submitted CADs will finely be compared to the real outcome of the 2-yr carcinogenicity studies. Main objective will be the assessment of accuracy of the predictions. This presentation will inform about the present details of this prospective analyses. Thus far 48 CADs have been collected, with more than 20 agreed virtual waivers. The presentation will discuss the strengths and weaknesses of the CADs, and the reasons why the DRA’s decided to deviate from the initial categorization by the sponsor.

2568 Lessons Learned from Completed Submissions: Case Studies
T. Bourcier, US FDA, Silver Spring, MD.

The prospective weight-of-evidence (WOE) assessment of carcinogenic risk and whether a 2yr rat study would add value to the WOE is documented in the carcinogenicity assessment document (CAD) substantially prior to the end of the rat study. Once submitted, the results of the 2yr rat Final Study Report (FSR) are compared to the assessment and predictions made in the associated CAD. An interim analysis of 14 CAD/FSR cases conducted in a joint session of the S1 expert working group determined that the WOE supporting a Category 3 designation was in fact reasonably consistent with the actual 2yr rat study outcome in this initial cohort of completed cases. Case studies will illustrate scenarios where Drug Regulatory Agencies and Sponsors both agreed and disagreed with the predicted value of the 2yr rat study for the assessment of carcinogenicity. Insights that emerge from these and other case studies will shape the scope and regulatory perspective of any alternative WOE approach the S1 Expert Working Group implements in the S1 guidelines.

2569 Leveraging New Capabilities to Optimize the Framework of Carcinogenicity Evaluation
F. D. Sistare, Merck & Co., Kenilworth, NJ.

Experience gained over decades of pharmaceutical rodent carcinogenicity testing has triggered a more flexible testing strategy proposal that if adopted will reduce animal testing burdens for drug candidates recognizable devoid of human carcinogenic risk. New challenges surface under such a flexible potential future framework that could reduce 2 yr rat carcinogenicity testing well beyond the anticipated 40% target. Use of the Tox2Flash2 model, and specific case applications of other genetically engineered rodents is anticipated to rise, as the need for 2 yr mouse study data also wanes. One underutilized pragmatic approach expected to gain momentum is more routine and early application of mechanism-based tissue carcinogenomic biomarkers.
to reliably discriminate human irrelevant from human relevant tumorigenic mechanisms, both of which oppose early histologic risk factors of neoplasia. Such a flexible framework for carcinogenicity testing will encourage deeper mechanistic insights from early shorter term conventional animal testing, will leverage new knowledge of cancer genetics and molecular pathway interactions to support target risk evaluations, and encourage earlier adoption and implementation of other emerging capabilities and endpoints to inform off-target toxicity potential. Examples will be presented describing practical applications of: (1) a routine early carcinogenic screening strategy in pharmaceutical development to alert and de-risk dose human relevant sustained off-target AhR activation while differentially informing rodent specific PPARα, CAR, PXR driven tumor mechanisms; (2) a genetically engineered rodent model applied early to address and avoid a theoretical target based carcinogenic risk concern, and (3) a strategic decision informed by a cancer genetic database.

2570 Application of Next-Generation Sequencing Approaches to Enhance Carcinogenicity Assessment of Pharmaceuticals In Vivo

M. Fielden, Amgen, Thousand Oaks, CA.

In addition to considering pharmacology, genetic toxicology, and chronic toxicology studies in the assessment of carcinogenic risk, ICH S1 also encourages the use of new biomarkers and technologies to aid in the overall assessment. Additional endpoints may be needed to de-risk target-based concerns or to assess in vivo translation of in vitro hazards. Due to the genetic basis of cancer we have focused on advancing DNA sequencing-based methods to inform carcinogenic mechanisms, including effects on genomic instability, in vivo mutagenesis and subclonal selection as early measures of preneoplasia. We have demonstrated the ability of Duplex Sequencing to identify chemical-induced mutagenesis in vivo, which permits evaluation of somatic mutagenesis in routine toxicity studies. In addition, we evaluated urethane-treated Tg.rasH2 mice for somatic mutations across a number of endogenous genes including the human HRAS transgene. Mutations in human codon 61 of HRAS, a well-known cancer driver, were detected as small subclones in the most cancer predisposed tissue (lung). These findings indicate the ability of Duplex Sequencing to identify rare variants that reflect the earliest stages of neoplastic evolution prior to overt lung tumor formation in a human-relevant cancer gene merely weeks after exposure. Whole-exome sequencing has also been used to evaluate translocations, copy number variants and insertions/deletions, which are well-established hallmarks of cancer not otherwise informed by standard assays. These alternative weight of evidence approaches are expected to reduce the uncertainty around potential cancer risk factors to help support risk categorizations that realize the benefits of the proposed ICH S1 modifications.

2571 The Chronic Cancer Bioassay Is Frequently Conducted for Pesticides When It Is Not Always Needed to Protect Human Health

D. C. Wolff, Syngenta, Research Triangle Park, NC.

A systematic approach that evaluates and integrates mechanism-based knowledge with exposure consideration allows for hazard characterization that are scientifically defensible and appropriate for regulatory decision-making of crop protection products. Using a problem formulation based and exposure driven evaluation strategy enables public health protective decisions to be made without unnecessary use of animals in large scale and redundant studies. Using the knowledge accumulated from the intended use, pesticide indication, and class of chemistry of the proposed pesticide will focus the questions that need to be answered to protect human populations from cancer risk. Investigation of the metabolic profile and subclonal selection as early measures of preneoplasia. We have demonstrated the ability of Duplex Sequencing to identify chemical-induced mutagenesis in vivo, which permits evaluation of somatic mutagenesis in routine toxicity studies. In addition, we evaluated urethane-treated Tg.rasH2 mice for somatic mutations across a number of endogenous genes including the human HRAS transgene. Mutations in human codon 61 of HRAS, a well-known cancer driver, were detected as small subclones in the most cancer predisposed tissue (lung). These findings indicate the ability of Duplex Sequencing to identify rare variants that reflect the earliest stages of neoplastic evolution prior to overt lung tumor formation in a human-relevant cancer gene merely weeks after exposure. Whole-exome sequencing has also been used to evaluate translocations, copy number variants and insertions/deletions, which are well-established hallmarks of cancer not otherwise informed by standard assays. These alternative weight of evidence approaches are expected to reduce the uncertainty around potential cancer risk factors to help support risk categorizations that realize the benefits of the proposed ICH S1 modifications.

2572 The Role of Dynamic RNA Modifications in Environmental Response and Disease

F. Tyson, NIEHS, Research Triangle Park, NC.

This symposium will discuss how dynamic RNA modifications can interpret environmental stimuli/challenges and respond by altering gene regulation, biological pathways, and disease outcomes. Chemical modifications of proteins, DNA, and RNA nucleoside moieties appear to have critical roles in regulating gene expression. These chemical modifications are central to the field of functional RNA modifications and emerging evidence suggests these modifications have critical roles in basic biological processes. These include: embryonic stem cell differentiation, excitotoxic cell death, development, intergenerational inheritance of acquired traits, regulation of RNA stability, temperature adaptation, meiotic progression, and regulation of RNA-RNA and RNA-protein binding interactions. A small number of covalent RNA modifications have been studied extensively, and recent evidence suggests that other newly discovered RNA modifications have interesting biological and disease functions in mammals. Moreover, recent studies have identified N6-methyladenosine (m6A) sites in thousands of human mRNAs, suggesting that this modification may play a role in regulation of alternative splicing and gene expression. The impact of the environment on chemical modifications of RNA molecules (the epitranscriptome) in the development of adverse human health outcomes is relatively unexplored. Technology advances in recent years have accelerated the detection of RNA modifications, and the RNA Modification Database currently lists approximately 100 RNA modifications identified in eukaryotic cells. This database also reveals transfer and ribosomal RNA are heavily modified, and many of these same modifications occur in messenger RNA and non-coding RNAs (including long non-coding and microRNAs). The function of most of the modifications found in messenger and non-coding RNAs remains a mystery, despite their potential to influence RNA properties and functions, including RNA stability, trafficking, localization, activity (enzymatic, sensing, or regulatory), and interactions with other molecules. This session will bring together toxicologists with investigators in the emerging area of functional RNA modifications to discuss the state-of-the-science as well as to identify research opportunities to interrogate how environmental exposures impact this layer of cellular regulation. Questions this session will address include: Have technologies for assessing RNA modifications matured enough to apply them to investigate how environment agents and exposures impact the role of functional RNA modifications and contribute to adverse health outcomes? Does the latest research suggest diverse environmental exposures can modify functional RNA modifications and/or the readers, writers, and erasers of these modifications? Was evidence presented that suggested or confirmed that stressors can have impacts on phenotypes through RNA modification mediated mechanisms? How can toxicologists leverage knowledge about epitranscriptomics to develop new biomarkers for toxicity or targets for therapeutic intervention?

2573 RNA Methylation in Gene Expression Regulation

C. He. University of Chicago, Chicago, IL. Sponsor: J. Goodrich

Over 100 types of post-transcriptional RNA modifications have been identified in all kingdoms of life. We have discovered the first two RNA demethylases, FTO and ALKBH5, which catalyze oxidative demethylation of the most prevalent modifications of mammalian messenger RNA (mRNA) and other nuclear RNA, N6-methyladenosine (m6A). These findings indicate that dynamic RNA modifications could impact biological regulation analogous to the well-known DNA and histone chemical modifications. We have also characterized proteins that selectively recognize m6A-modified mRNA and affect the translation status and lifetime of the target mRNA, as well as molecular machines that deposit the m6A methylation on mRNA. Functional studies reveal m6A methylation as a fundamental mechanism to synchronize groups of functional RNA modifications and emerging evidence suggests these modifications have critical roles in basic biological processes. These include: embryonic stem cell differentiation, excitotoxic cell death, development, intergenerational inheritance of acquired traits, regulation of RNA stability, temperature adaptation, meiotic progression, and regulation of RNA-RNA and RNA-protein binding interactions. A small number of covalent RNA modifications have been studied extensively, and recent evidence suggests that other newly discovered RNA modifications have interesting biological and disease functions in mammals. Moreover, recent studies have identified N6-methyladenosine (m6A) sites in thousands of human mRNAs, suggesting that this modification may play a role in regulation of alternative splicing and gene expression. The impact of the environment on chemical modifications of RNA molecules (the epitranscriptome) in the development of adverse human health outcomes is relatively unexplored. Technology advances in recent years have accelerated the detection of RNA modifications, and the RNA Modification Database currently lists approximately 100 RNA modifications identified in eukaryotic cells. This database also reveals transfer and ribosomal RNA are heavily modified, and many of these same modifications occur in messenger RNA and non-coding RNAs (including long non-coding and microRNAs). The function of most of the modifications found in messenger and non-coding RNAs remains a mystery, despite their potential to influence RNA properties and functions, including RNA stability, trafficking, localization, activity (enzymatic, sensing, or regulatory), and interactions with other molecules. This session will bring together toxicologists with investigators in the emerging area of functional RNA modifications to discuss the state-of-the-science as well as to identify research opportunities to interrogate how environmental exposures impact this layer of cellular regulation. Questions this session will address include: Have technologies for assessing RNA modifications matured enough to apply them to investigate how environment agents and exposures impact the role of functional RNA modifications and contribute to adverse health outcomes? Does the latest research suggest diverse environmental exposures can modify functional RNA modifications and/or the readers, writers, and erasers of these modifications? Was evidence presented that suggested or confirmed that stressors can have impacts on phenotypes through RNA modification mediated mechanisms? How can toxicologists leverage knowledge about epitranscriptomics to develop new biomarkers for toxicity or targets for therapeutic intervention?
N6-methyladenosine (m6A) is an abundant, reversible chemical modification that regulates function and stability of many types of RNAs. We use biochemical and structural methods to elucidate how m6A marks are generated by RNA methyltransferases. We show that Metl3 and Metl14 cooperate to recognize and catalyze modification of target RNAs. Metl3 is the catalytically active enzyme, while Metl14 has a structural role to support the necessary conformation and to bind the substrate RNA. We also show that Metl16, another m6A writer, uses a distinct mechanism to recognize and bind the RNA substrates. We determine the molecular basis for how the RNA methyltransferases manifest distinct substrate specificities. Moreover, we show that a polypeptide in the catalytic domain of METTL16 can undergo a conformational change, and that it can act as an autoregulatory switch for the methyltransferase activity. Furthermore, we reveal how disease mutations of m6A writers lead to aberrant methyltransferase activity. Together, our study shows that each m6A writer enzyme has evolved to modify a specific set of RNAs, with controlled efficiency for the specific RNA/enzyme combination. Exposure to certain harmful chemicals such as arsenic has been shown to have an impact on the methylation levels of DNA and proteins, as well as changing intracellular SAM levels. Since our model of METTL16 activity directly links intracelular SAM levels to RNA methylation, our mechanistic studies may also provide insight into how certain environmental factors might have a widespread impact on the epitranscriptome.

Once deemed heretical, the idea of "inheritance of acquired characteristics" is now supported by increasing evidence from multiple species, including mammals. We showed that paternally acquired traits (e.g. metabolic disorders induced by high-fat diet, HFD) can be "memorized" in the sperm, encoded in the form of RNAs and RNA modifications, and transmit paternally acquired phenotypes to the offspring via shaping early embryo development. We previously discovered that sperm RNAs, particularly 30-40nt RNA fractions that enriched with tRNA-derived small RNAs (tsRNAs), can act as epigenetic factors in mediating intergenerational inheritance of acquired traits, e.g., paternal metabolic disorders and trauma induced stress phenotypes. Our latest research further showed that deletion of a mouse tRNA methyltransferase, Dnm2, abolished sperm tsRNA-mediated transmission of HFD-induced metabolic disorders to offspring. Dnm2 deletion prevented the elevation of RNA modifications (mSc, m2G) in sperm 30-40nt RNA fractions that are induced by HFD. Also, Dnm2 deletion altered the sperm small RNA expression profile, including levels of tsRNAs and tRNA-derived small RNAs (tsRNAs), which may resemble the presence in composing a sperm RNA-binding signature that is needed for paternal epigenetic memory. Finally, we showed that Dnm2-mediated mSc contributes to the secondary structure and biological properties of tsncRNAs, implicating sperm RNA modifications as an additional layer of paternal hereditary information.

A major adaptive cellular response to environmental stress is the formation of stress granules. Stress granules are RNA-protein assemblies that function, in part, by sequestering specific cellular mRNAs. However, the specific mRNAs that are targeted to stress granules are not fully understood. Here we show that diverse types of environment stress, including UV, heat shock, arsenite, and endoplasmic reticulum stress elicited by thapsigargin all induce the formation of stress granules that are enriched in m6A RNAs with the modified nucleotide N6-methyladenosine (m6A). Using a transcriptomic analysis of stress granules, we find that mRNA that lack m6A are largely excluded from stress granules, while the enrichment of mRNAs in stress granules is directly proportional to the number of m6A in an mRNA. The enrichment of m6A mRNA is seen in stress granules irrespective of the type of stress used to elicit stress granule formation. The stress granule-targeting effect of m6A is mediated by its binding to the YTHDF family of m6A-binding proteins. We find that YTHDF partition into stress granules elicted by UV, heat shock, arsenite, and endoplasmic reticulum stress. Furthermore, we find that the sequestration of m6A mRNAs results in their preferential translational suppression during stress. Moreover, the significance of m6A mRNAs and YTHDF mRNAs in stress granules is demonstrated by experiments in which we depleted either m6A or YTHDF proteins from cells. In these cells, stress granule formation was markedly impaired. Overall, these data reveal that a fundamental role of stress granules elicited by diverse toxic insults is to sequester m6A mRNAs and control their translation during and after stress. Our data support the idea that selective control of m6A mRNAs is critical for the proper control of the translational program during and after stress.

Epitranscriptomic signals regulate gene expression by altering RNA structure and stability, with some tRNA modifications directly linked to translational regulation. We used systems based approaches to demonstrate that prokaryotic and eukaryotic cells respond to stress and toxicants by enzymatically reprogramming the levels of modified nucleosides (5'-oxacytidyluridine (cm5OSU), 5'-methyl-5'-O-methyl-2'-G-methyluridine (cm5Gm)), and more) in tRNA, to generate unique epitranscriptomic signatures representing responses to specific toxicants. Further we demonstrated that stress induced changes in the levels of specific modified uridines and cytidines in the anticondon of tRNA translationally regulate codon-biased transcripts. Example systems from Saccharomyces cerevisiae, human cells and mice demonstrate that the absence of specific RNA modifications and the enzymes that catalyze their incorporation into tRNA corrupts the responses to DNA damage and reactive oxygen species (ROS). Notably, we developed a transgenic mouse that is deficient in the tRNA methyltransferase Alkbh8 which is required for the formation of S-methylthiocarbamoyluridine (mcm5SU) and mcm5Um on tRNA for selenocysteine. These modifications are utilized during the process of stop codon recoding to allow for the incorporation of selenocysteine into selenoproteins (i.e. glutathione peroxidases and thioredoxin reductases) that play critical roles in the detoxification of ROS. We have shown that Alkbh8/-/- cells have decreased levels of selenoproteins, are sensitized to damage by external agents (H2O2, rotenone and naphthalene) that promote an increase in ROS and that Alkbh8/-/- lung tissue has exposure induced pathologies. Further, to compensate for the epitranscriptomic deficiency, Alkbh8/-/- cells and lung tissue upregulate complimentary stress response and damage mitigation systems. Results from our Alkbh8/-/- mouse model highlight how tRNA epitranscriptomic systems are up-regulated in response to environmental stressors to protect cells and lung tissue. The broad impact of epitranscriptomic systems in toxicology is only beginning to be realized as they regulate gene expression and control the synthesis of stress response proteins. Further epitranscriptomic responses have the potential to be used as biomarkers of response or exploited for pharmaceutical applications.

Unexpected ocular findings in general toxicology studies represent a unique challenge in pharmaceutical drug development. Eye lesions are uniquely monitorable in this setting, often using the same techniques that are applied in clinical studies. This allows for detailed assessment of the dose response and onset of lesions as well as their functional impact. However, understanding the specialized grading scales and imaging modalities is key to interpreting these data. Additionally, many tissues of the eye, particularly the retina, are terminally differentiated and unable to regenerate following toxic insult. This makes the detection, characterization, and mechanistic understanding of ocular lesions caused by novel therapeutics of vital importance for an assessment of human risk prior to clinical trials. The objective of this session will be to focus on adverse ocular findings on general toxicology studies for non-ophthalmology products. The first presentation will focus on the common ocular assessments used on these studies and frequently observed ocular lesions. This will be followed by three case studies in which unexpected ocular findings were observed on general toxicology studies, with a focus on the sponsor’s investigative and regulatory strategy to address these findings. Finally, a speaker from the US FDA will provide a perspective on regulatory expectations for therapies with adverse ocular effects.

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While the eye represents arguably the most elegantly complex and specialized structure in the biologic system, its unique morphologic and physiologic features also puts it at risk. From front to back, the eye comprises a variety of metabolically-active tissues supported by a robust blood supply, with the retina maintaining one of the highest metabolic demands of any mammalian tissue. Despite autoregulatory mechanisms and immunologic specializations that aim to protect the eye from endogenous harm, it remains highly susceptible to exogenous toxicity caused by, among other agents, systemically-administered drugs. These toxicities may be just as variable in their presentations as the tissues comprising the eye, ranging from minor and irreversible irritations involving the ocular surface, to severe and vision-threatening effects involving the retina. Given these risks, thorough assessment of the eyes in preclinical animal models is essential and must be rigorous in order to ensure the safety and well-being of animals and human patients, alike. This lecture will introduce and discuss the requisite components of the ophthalmic examination in general toxicology studies, including the necessary instrumentation, techniques, and procedures that ensure a complete ocular assessment. The importance of precise and consistent semi-quantitative scoring (grading) of ocular findings will also be discussed. Finally, a survey of the most frequent and clinically relevant background findings in common laboratory species will be presented, with a focus on their impact on interpretation of toxicology findings.

Antibody drug conjugates (ADCs) are a promising class of cancer therapeutics that takes advantage of the antibody (Ab) specificity to target tumor cells and the strong potency of small molecule cytotoxic drugs. Despite early clinical success and marketing approvals of Adcetris® and Kadcyla®, the clinical development of this class of cancer therapeutics continues to be hindered by safety issues. The majority of the clinical safety data available is with microtubule inhibitor (MTI) containing ADCs, which include the auristatins monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF), and the maytansine derivatives, DM1 and DM4. Myelosuppression and peripheral neuropathy are the most common clinical toxicities with MTIs as a consequence of targeting the rapidly dividing cells of the bone marrow and inhibiting axonal transport critical for the long projecting peripheral nerves, respectively. However, ocular toxicity is another challenging clinical safety issue with MTI containing ADCs and in some cases the dose limiting toxicity. The ocular toxicity in patients manifests as keratitis, blurry vision and dry eye, and is not consistently seen in nonclinical toxicity studies. The assessment of ocular toxicity with MTI ADCs and specific case examples of the nonclinical to clinical translatable ability will be discussed in this talk.

Ocular Toxicity with Microtubule Inhibitor Containing Antibody Drug Conjugates: Nonclinical to Clinical Translatability Case Examples

Lessons Learned in How to Manage Ocular Findings: A Case Study

Can We Panelize Seizure?
by inexperienced operators. Thus, confirmation of drug-induced seizure or seizure-like activity requires a follow-up electroencephalogram (EEG) study. Some progress has been made in in-life detection of seizure using automated video systems that record and analyze animal movements, looking for abnormalities. Nonetheless, it would be far preferable to have an earlier prediction of seizurogenic risk that could be used to eliminate liabilities early in discovery while there are still options in chemistry. Two approaches offer exciting opportunities. Microelectrode array is now able to detect seizurogenic signals in iPSC-derived cortical neural stem cells differentiated to electrically active cortical neurons. This offers great potential to screen for seizurogenic liability in an in vitro system. A second approach could be based on an understanding of the neuronal ion channels implicated in the seizurogenic response. There is clear evidence for the involvement of ion channels in seizure. Genetic studies have pointed to a role for voltage-gated sodium and potassium channels and the ligand-gated ion channels, GABA-A and nicotinic acetylcholine receptors. Pharmacologically, a number of ion channel modulators are known to be seizurogenic, such as Chlorpromazine. Recently, great progress has been made in developing these in vitro seizure models and in characterizing the ion channels both at the expression level and at the functional level. These data may provide the opportunity to panelize seizure by creating a panel of ion channel assays that predict seizure, linked to an in vitro assay, with the ultimate aim of eliminating compounds predicted to be associated with the pro-seizurogenic state. The regulatory community has great expertise and experience of how to shape such work for decision-making from lessons learned from implementation of similar approaches in derisking cardiovascular liability. This session will address the issue, the basic science, and the regulatory context. It is relevant to academia, industry, CROs, and regulators who wish to learn more about these exciting, new, and high-impact developments in drug discovery and development.

**2585 Seizure Liability in Drug Discovery and Development: Current State of Play**

J. Valentin, UCB, Braine-l’Alleud, Belgium.

Drug-induced convulsions or seizures are a serious, potentially life-threatening adverse drug reaction that can result in failure of drugs to be licensed for use, withdrawn from the market or labelled as such. Compounds associated with this liability span a wide variety of pharmacological classes and therapy areas, including many not targeted at the central nervous system. Assessing the risk of seizurogenic and non-seizurogenic liabilities in the drug discovery process using low-throughput, resource intensive in vivo behavioral models. For example, one known CNS active compound was associated in preclinical species with convulsions confirmed as being seizures. The overall risk assessment did allow to progress into Phase I clinical trials with appropriate safety monitoring (incl. EEG) but several adverse events (syncpe, clinically observable seizure, asymptomatic EEG spikes) were noted at exposures below where seizure was seen preclinically, halting the clinical trial. In a second example from a neuroscience project, candidate compounds induced convulsions in rodent and non-rodent species, at low exposure levels. All compounds inducing convulsions in vivo showed a strong inhibitory activity on GABA-A channels, whereas compounds with mild or no inhibitory effect did not induce seizures supporting the hypothesis of an off-target mechanism. In EEG experiments, convulsions were always preceded by premonitory clinical signs and abnormal EEG, confirming their CNS origin. The lead compounds in this project were discontinued. These examples illustrate how current approaches do not allow sufficient time to mitigate risk by influencing chemical design. Early identification using cheaper, higher throughput and predictive assays with lower animals and compound requirements would be preferable. Over the last decade, several assays that are compatible with the design-make-test-analyze cycle have become available. In silico, in vitro and in vivo assays may be combined in a rational step-wise cascade to allow more effective i) hazard detection and elimination in hit identification and lead optimization, ii) risk assessment on drug candidates taking into account the safety margin, therapeutic indication and patient population and iii) risk mitigation and management in clinical development with appropriate monitoring and rescue therapies.

**2586 Identification and Confirmation of Seizure Liability In Vivo: The Importance of Behavioral Monitoring and EEG Recording**

S. Authier, Citoxlab North America, Laval, QC, Canada.

Although it would be advantageous to identify seizure risk in the early phases of drug discovery, ideally using in vitro methodologies, currently this risk is generally first detected in in vivo studies later in the drug discovery process. The first indication of a potential seizure liability is often CNS clinical signs such as tremor or abnormal movements which may not result from seizure activity, in addition tonic-clonic convulsions can be incorrectly diagnosed by non-expert observers. To confirm the presence of drug-induced seizure, an electroencephalogram (EEG) study is required. In addition, due to the low frequency of such clinical observations in traditional toxicology studies, these events can often be missed in short term studies and are not detected until chronic studies much later in drug development. For these reasons, it is helpful to obtain a full description of behavioural findings when assessing seizure risk. One way this can be achieved is by using continuous video monitoring, ideally combined with EEG recordings so both sets of data can be fully integrated. Overall, the in vivo toxicity testing paradigm aims i) establish the exposure to effects relationship; ii) characterize the convulsions, iii) detect premonitory signs that could be monitored in clinical setting, and iv) assess the development of potential tolerance or sensitization after repeat dosing and underlying seizure liability mechanisms.

**2587 Development of Seizure Prediction Methods Using MEA System in Human iPSC-Derived Neurons**

I. Suzuki. Tohoku Institute of Technology, Tohoku, Japan. Sponsor: J. Pierson

Functional evaluation assays using multi-electrode array (MEAs) in cultured hiPSC-derived neurons are being developed to predict the convulsive potential of new drugs. Dose-dependent firing data of more than 20 convulsive drugs and non-convulsant drugs were obtained. A principal component analysis approach coupled with artificial intelligence (AI), convulsive and non-convulsive drugs were separated and classified using effective parameter sets. AI extracted 4096 feature quantities from the training set of image data of firing patterns; AI analysis of the MEA data from the test set provided accurate answers on drug name and concentration. Furthermore, a comparison of in vitro results with in vivo human ECoG convulsive responses, showed that the characteristic in vivo convulsive firing enhancement of the high gamma wave and beta wave was observed in in vitro hiPSC-derived neurons. Some caution should be used in the interpretation of hiPSC-derived data for predicting seizure in the clinical setting: are the test cells expressing the correct ion channels and signaling molecules to allow a phenotypic response? Also, how representative are these cells of adult neurons? Since data are usually derived from one or maybe two or three human donors with their individual genetic fingerprint, how general are the results for the broader population? Coupled with this, AI is an emerging technique with its own challenges; how well do we understand the underlying data processing and thus the interpretation? Despite these ongoing discussions, in summary our data show that MEA measurement of hiPSC-derived neurons coupled with our analysis methods appear to be effective for the prediction of convulsion toxicity and their mechanisms of action, as well as the comparison of convulsions induced in vivo.

**2588 Using Ion Channels to Panelize Seizure: Where Are We Up To?**

M. Morton. ApconIX, Alderley Edge, United Kingdom. Sponsor: J. Pierson

There is clear evidence for the involvement of ion channels in seizure. Genetic studies have pointed to a role for voltage-gated sodium and potassium channels and the ligand-gated ion channels, GABA-A and nicotinic acetylcholine receptors. Pharmacologically, a number of ion channel modulators are known to be seizurogenic such as Chlorpromazine. Should we therefore be looking to screen compounds in early discovery against a panel of ion channels, and what would this panel look like? Ion channel screening against a panel of ion channels has been successfully employed for decades to reduce cardiovascular safety liability. Routine screening against the cardiac potassium, sodium and calcium channels, and optimization of medicinal chemistry away from these liabilities, has reduced the number of drug withdrawals due to hERG/QT prolongation. Coupled with an informatics approach, screening for cardiovascular liability in entering a new paradigm with the advent of the comprehensive in vitro proarhythmic assessment (CiPA). Can the same approach be applied to reducing seizure liability? The challenges include the complexity of CNS pharmacology along with the more practical challenges of identifying drugs and non-convulsant drugs were obtained using MEAs. Using a principal component analysis approach coupled with artificial intelligence (AI), convulsive and non-convulsive drugs were separated and classified using effective parameter sets. AI extracted 4096 feature quantities from the training set of image data of firing patterns; AI analysis of the MEA data from the test set provided accurate answers on drug name and concentration. Furthermore, a comparison of in vitro results with in vivo human ECoG convulsive responses, showed that the characteristic in vivo convulsive firing enhancement of the high gamma wave and beta wave was observed in in vitro hiPSC-derived neurons. Some caution should be used in the interpretation of hiPSC-derived data for predicting seizure in the clinical setting: are the test cells expressing the correct ion channels and signaling molecules to allow a phenotypic response? Also, how representative are these cells of adult neurons? Since data are usually derived from one or maybe two or three human donors with their individual genetic fingerprint, how general are the results for the broader population? Coupled with this, AI is an emerging technique with its own challenges; how well do we understand the underlying data processing and thus the interpretation? Despite these ongoing discussions, in summary our data show that MEA measurement of hiPSC-derived neurons coupled with our analysis methods appear to be effective for the prediction of convulsion toxicity and their mechanisms of action, as well as the comparison of convulsions induced in vivo.
D. Mellon. US FDA, Silver Spring, MD. Sponsor: J. Pierson

Convulsions pose a major challenge in drug development and their observance in in vivo studies is often a limiting factor in preclinical results in humans. Kindled seizures occurring in repeat dose toxicology studies late in development present even greater challenges which can derail a development program. Current approaches are limited to careful monitoring and use of EEG studies to understand risk; however, given potential for low frequency events, additional alternative assessments continue to assist in evaluation. Panelizing seizure risk early in development could identify compounds with greater risk early and avoid wasting resources. These assays could also have utility at later stages in development to help a weight-of-evidence approach in assessing the implications for human risk assessment of convulsions observed in toxicology studies and how to compare relative risk across different therapeutic options. However, we argue that the best way to avoid being able to use these approaches for regulatory decision making. The major challenges are in validating the predictivity of the available assessment tools which will require identification of appropriate endpoints and thresholds of concern derived from these data, standardizing methodologies, and building the database of in vitro and in vivo data to demonstrate reliability in their predictive capacity to inform the overall risk-benefit profile. Specifically, there needs to be scientific consensus on the channels involved in seizure and on how to measure the effect of potential new drugs on the function of these ion channels. Based on experience with panelizing cardiac arrhythmia via the GIPA, correlation of ion channel function with associated phenotype in vitro coupled with methods of data capture and processing will provide confidence in moving these methods forward into the regulatory framework.

A. Vale. University of Birmingham, United Kingdom.

Nerve agents are chemically related to organophosphorus insecticides and have a similar mechanism of toxicity, but their human acute toxicity is considerably greater, particularly via the dermal route. They act by inhibiting the enzyme acetylcholinesterase (AChE) which is responsible for the deactivation of acetylcholine (ACh) at neuromuscular junctions and at synapses in the central and peripheral nervous systems. In addition, the process of "aging" results in a monoalkyl product which does not reactivate spontaneously and cannot be reactivated by pyridinium oximes, such as pralidoxime and obidoxime. Nerve agents were employed most recently in an attack on Khan Sheikhoun, Syria, in April 2017. VX was used as a weapon of assassination on February 13, 2017, when Kim Jong-nam, was killed at Kuala Lumpur International Airport. In Salisbury, England, on March 4, 2018, Sergei Skripal, his daughter Yulia, and a policeman investigating the incident were severely poisoned following exposure to a Novichok agent. Subsequently, on June 30, 2018, two more individuals were severely poisoned with the same Novichok agent, one of whom died. All these releases indicate that countries and their clinicians must be prepared adequately to treat casualties optimally from nerve agent exposure. This requires an understanding of the mechanisms of toxicity of these agents, the factors that influence their clinical impact, and knowledge of potential treatments. Although the signs and symptoms manifested by exposed individuals will aid diagnosis, reliable point-of-care diagnostic systems will expedite triage and the application of appropriate medical countermeasures. Most of these systems are based on measurement of acetyl- or butyrylcholinesterase activity, but more recently an easy-to-use lateral flow assay has been developed that can be used for both rapid point-of-care diagnosis, as well as for detection of submicrogram amounts of nerve agents in/on various matrices. However, a useful verification of an exposure requires a variety of specialized techniques, and the utility of these methods will be exemplified by the analysis of various samples from the Syrian Arab Republic conflict in April 2013. Much research is underway to improve the current treatment regimens, which include an anticholinergic drug (e.g., atropine) to antagonize the effects of excess ACh at muscarinic effector sites, the use of an oxime (e.g., pralidoxime, obidoxime, and HI-6) to reactivate nerve agent-inhibited AChE, and a benzodiazepine to prevent or stop nerve agent-induced seizures. Four innovative treatment approaches will be described during the session. First, the development of catalytic scavengers: multiple candidate enzymes on differential expression levels have been expressed in a cell line coupled with a single enzyme capable of catalyzing the hydrolysis of a broad spectrum of organophosphorus (OP) compounds into nontoxic products. The most promising candidate enzyme platform is the bacterially produced recombinant variant of organophosphorus hydrolase from B. diminuta. In vivo studies, two different organo-phosphorus hydrolase variants were capable of providing protection against at least 2 x LD50s of all of the OPs tested. Second, a series of novel substituted phenoxylkyl pyridinium oximes have been produced, which reduce brain AChE inhibition in rats treated with high-dose challenges of OP compounds. These novel oximes also have shortened the time to cessation of OP-induced seizure-like behavior on the day of OP challenge and have reduced neurodegeneration observed four days after the challenge by such neural markers as NeuN. These oximes when delivered intramuscularly show a high ability to provide 24-hour survival from lethal OP dosages and they have a half-life of 2.5 hours or greater in the blood stream of the rat, and therefore have promising pharmacokinetics. Third, the use of the bispyridinium non-oxime compound MB327 increased the survival of rats poisoned with soman, without reactivation of AChE. Moreover, it has been shown in human and rodent muscle tissue that paralysis of the respiratory muscles could be restored partially by MB327. In addition, MB327 and several structurally analogous compounds were able to restore function of nicotinic receptors (Torpedo californica muscle-type and human α7 subtype) after desensitization (demonstrated with electrophysiological techniques using patch clamp and SSM-based electrophysiology). Moreover, molecular modeling allowed identification of a new allosteric binding site close to the transmembrane domain of the nicotinic receptor. From these data, it may be concluded that by using MB327 and its analogues as a template, new structures with improved binding properties may be able to antagonize paralysis of blocked muscle function, without reactivation of inhibited AChE. Fourth, improvement of nerve agent elimination by small molecule scavengers might further contribute a beneficial effect. Indeed, modified cycloexetrins are able to bind the highly toxic (-)-isomere of GF and calixarenes are able to enhance degradation of VX by a factor of 3500.
Poisoning by organophosphate (OP) anticholinesterases can result in a variety of signs of toxicity including seizures which, if prolonged, can lead to permanent brain damage. An important therapeutic need to combat OP poisoning has long been a brain-penetrating reactivator of inhibited acetylcholinesterase (AChE) that could assist in preventing or attenuating the seizure-induced damage. Because the typical pyridinium oxime reactivators, such as 2-PAM and HI-6, bear a permanent positive charge, they are not effective at reversing brain AChE inhibition and promoting neuroprotection because of their very limited ability to cross the blood-brain barrier. Several laboratories have pursued strategies to allow reactivators to enter the brain through either non-oxime structures or modified oxime structures, and some of these strategies have met with success. Our laboratories have developed a platform of novel substituted phenoxyalkyl pyridinium oximes that have catalytic activity. Formulation of the enzymes to promote circulatory stability and brain penetration of the enzymes to provide protection against all G- and V-type OP nerve agents. The most promising candidate enzyme platform is the bacterially produced recombinant variant of organophosphorus hydrolase (OPH) from B. diminuta. In vivo protective efficacy of candidate OPH scavengers as prophylactics was tested in guinea pigs by administering the enzyme via a carotid catheter (iv), followed 20 minutes later by subcutaneous injection of increasing doses of the OP nerve agents GA, GB, GD, GF, VX, VR, or VM. A stage-wise, adaptive dosing experimental design was used to determine the median lethal dose (LD50) of each OP in the context of enzyme prophylaxis. We report that a combination of two different OPH variants, administered at 5 mg/kg of each enzyme, is capable of providing protection against at least 2 LD50 of the OP compound tested. The results indicate that broad spectrum prophylactic protection against OP intoxication can be provided with a cocktail of two different catalytic scavengers with appropriate catalytic activity. Formulation of the enzymes to promote circulatory stability will also be discussed. This work was supported by the NIH CounterACT Center of Excellence grant USA5 NS058183 (to D.M.C.) and by the Defense Threat Reduction Agency-Joint Science and Technology Office, Medical S&T Division.

Electronic cigarettes (e-cigs) are vehicles for inhaled delivery of nicotine and are considered by some to be smoking cessation aid devices, although they are not approved by US FDA for cessation treatment, nor even considered as nicotine replacement therapy (NRT). The benefits of e-cigs as nicotine replacement vehicles are countered by an expanding body of knowledge regarding potential health hazards and social consequences. E-cig aerosols have a unique physical and chemical composition that is different from that of cigarette smoke, although both aerosols contain nicotine and other similar toxicants. At present, there are very limited regulatory or legal processes overseeing the production and sales of e-cig devices. Thus, the general safety of e-cigs regarding human health is still a matter of controversy. According to the CDC, e-cig use among high school students increased by more than 300% between 2013 and 2014, and its use has continued to increase. The 2016 Surgeon General’s report labeled e-cig use among adolescents and young adults a major public and societal health concern. The public perception of e-cigs as a healthier alternative to conventional cigarettes has led to the recent rise of e-cig use among youth and young adults, including women of childbearing age. Although research is emerging around e-cigs in general, there continues to be a lack of scientific evidence regarding the safety and risks of e-cig use on maternal and fetal health; however, adverse health effects of nicotine on maternal and fetal outcomes are well documented. In the US, more than 10% of women smoke during pregnancy, and recent national surveys show that this proportion is similar for the use of e-cig devices. Controversy, however, over e-cig use during pregnancy is being hotly debated between US and UK regulators. Currently, there are no guidelines for use of e-cigs during pregnancy. This highlights the urgent need to bridge the clinical and scientific knowledge gap related to e-cig use during pregnancy. Whether e-cig use during pregnancy is safer and represents a better risk-to-benefit ratio for the developing fetus than conventional cigarettes is unclear due to lack of studies. This session provides a unique focus on the knowns and unknowns of e-cig use during pregnancy, including ethical and societal implications. A panel discussion incorporating both US and UK basic research and policy frameworks will represent a strong base for discussing whether e-cig use could be a reasonable alternative for pregnant smokers who are otherwise unable to quit.
Electronic cigarettes or Electronic Nicotine Delivery Systems (ENDS) are widely available in almost unlimited variety, including different flavors, nicotine concentrations, propylene glycol and glycerin ratios, and battery options. With finalization of the ‘deeming’ rule on August 8, 2016, US FDA Center for Tobacco Products (CTP) regulates products that meet the regulatory definition of a tobacco product, including ENDS. Tobacco product regulation uses a public health standard – not the traditional ‘safety and efficacy’ standard used for other US FDA approved products. This public health standard requires US FDA to consider the impact of any tobacco product regulatory decision on the entire population – including current tobacco users, former users, users who may be trying to quit, nonusers, and youth and other vulnerable populations. Although current evidence suggests that ENDS may be less harmful than cigarettes for smokers who completely switch, the health effects are not well characterized and long-term data are not available. The health risks associated with ENDS are likely dependent on the specific device or e-liquid being used and on user behavior. There are limited data on the chemical constituents in the aerosols and the effects of acute/short-term exposure. Additionally, the effects of repeated inhalation of aerosolized chemicals, including propylene glycol, glycerin, and flavorings is unknown. Nicotine is known to have cardiovascular effects (increasing heart rate and blood pressure) and adverse impacts on maternal and fetal health during pregnancy including an adverse impact on the developing fetal brain. There is no amount of nicotine exposure that is considered safe during pregnancy. US FDA is interested in understanding the short and long-term health effects of ENDS use in both users and non-users of tobacco. Additional information will better inform the development of policy options and assist in evaluation of the impact of US FDA’s regulatory efforts.

Electronic Nicotine Delivery Systems in Context: The US FDA Perspective
P. Hung. US FDA, Silver Spring, MD. Sponsor: A. Noel

Application of In Vitro Approaches for the Assessment of Next-Generation Tobacco and Nicotine Products: A Tobacco Company Perspective

There has been a significant increase in the use of next generation tobacco and nicotine products (NGPs), including e-cigarettes. Developing toxicological screens for consumer safety across the wide range of e-cigarette devices and liquids has become particularly important. The use of in vitro assays, modelling key tobacco-disease related endpoints may be suitable for NGP assessment. We will present the challenges and opportunities offered using in vitro approaches. Cellular exposure and test article generation principles will be outlined, in particular exploring the generation, dilution and delivery of aerosols to in vitro cellular systems, and exposure considerations. A number of assays will be explored including classical in vitro toxicological assessing mutagenicity and cytotoxicity, showing greatly reduced responses from NGPs relative to cigarettes. Human cellular-based in vitro assays incorporating continuous cell lines to more complex organotypic reconstituted tissue systems, integrating adverse outcome pathways and next generation technology will be presented. Data from in vitro can support the risk assessment of e-cigarettes as part of a larger weight-of-evidence and reinforce the potential of e-cigarettes to play an important role in tobacco harm reduction. This is particularly needed in the context of pregnancy where women are unable to quit smoking.

Electronic Nicotine Delivery Systems in Context: The United Kingdom

Sponsor: A. Noel

E-cigarettes in Pregnancy: Current Evidence and Recommendations for Practice in the United Kingdom
F. Bates. The Lullaby Trust, London, United Kingdom. Sponsor: A. Noel

In the United Kingdom (UK) about 2.9 million people use electronic cigarettes (e-cigs, vapes, and electronic nicotine delivery systems [ENDS]) for recreational purposes or as a smoking cessation aid. However, the public health community remains divided concerning the appropriateness of endorsing devices whose safety and efficacy for smoking cessation remain unclear; thus, ethical dilemmas arise concerning product safety, efficacy for smoking cessation and reduction, use among adult non-smokers, use among youth, use in public places and marketing. Perhaps one of the most challenging current ethical quandaries is the use of e-cigs during pregnancy. While pregnancy provides motivation for women to quit smoking, in 2016 in England, for example, there was only a 0.1% decrease in the prevalence of women who smoked during their pregnancy. The health effects related to maternal tobacco smoking have been widely studied and reported, whereas the evidence of short- and long-term effects of inhaled e-cig aerosols on pregnancy outcomes and on the health of offspring is largely unknown. In recent years e-cigs have become the most popular aid for smoking cessation in the UK and a significant proportion (16%) of pregnant women report using them during pregnancy. Due to these patterns of use and remaining challenges relating to reducing smoking in pregnancy, a multi-agency group comprises of some of the main health professional and research organizations, along with health charities and mother and baby organizations in England (the Smoking in Pregnancy Challenge Group) have developed practical guidance on vaping during pregnancy. This proposes a positive policy framework related to e-cig use during pregnancy for women who cannot quit smoking. Here, we will discuss the current evidence and recommendations for practice in the UK.

Electronic Nicotine Delivery Systems in Context: The US FDA Perspective
P. Hung. US FDA, Silver Spring, MD. Sponsor: A. Noel

Application of In Vitro Approaches for the Assessment of Next-Generation Tobacco and Nicotine Products: A Tobacco Company Perspective

There has been a significant increase use of next generation tobacco and nicotine products (NGPs), including e-cigarettes. Developing toxicological screens for consumer safety across the wide range of e-cigarette devices and liquids has become particularly important. The use of in vitro assays, modelling key tobacco-disease related endpoints may be suitable for NGP assessment. We will present the challenges and opportunities offered using in vitro approaches. Cellular exposure and test article generation principles will be outlined, in particular exploring the generation, dilution and delivery of aerosols to in vitro cellular systems, and exposure considerations. A number of assays will be explored including classical in vitro toxicological assessing mutagenicity and cytotoxicity, showing greatly reduced responses from NGPs relative to cigarettes. Human cellular-based in vitro assays incorporating continuous cell lines to more complex organotypic reconstituted tissue systems, integrating adverse outcome pathways and next generation technology will be presented. Data from in vitro can support the risk assessment of e-cigarettes as part of a larger weight-of-evidence and reinforce the potential of e-cigarettes to play an important role in tobacco harm reduction. This is particularly needed in the context of pregnancy where women are unable to quit smoking.

Electronic Nicotine Delivery Systems in Context: The United Kingdom

Sponsor: A. Noel

E-cigarettes in Pregnancy: Current Evidence and Recommendations for Practice in the United Kingdom
F. Bates. The Lullaby Trust, London, United Kingdom. Sponsor: A. Noel

In the United Kingdom (UK) about 2.9 million people use electronic cigarettes (e-cigs, vapes, and electronic nicotine delivery systems [ENDS]) for recreational purposes or as a smoking cessation aid. However, the public health community remains divided concerning the appropriateness of endorsing devices whose safety and efficacy for smoking cessation remain unclear; thus, ethical dilemmas arise concerning product safety, efficacy for smoking cessation and reduction, use among adult non-smokers, use among youth, use in public places and marketing. Perhaps one of the most challenging current ethical quandaries is the use of e-cigs during pregnancy. While pregnancy provides motivation for women to quit smoking, in 2016 in England, for example, there was only a 0.1% decrease in the prevalence of women who smoked during their pregnancy. The health effects related to maternal tobacco smoking have been widely studied and reported, whereas the evidence of short- and long-term effects of inhaled e-cig aerosols on pregnancy outcomes and on the health of offspring is largely unknown. In recent years e-cigs have become the most popular aid for smoking cessation in the UK and a significant proportion (16%) of pregnant women report using them during pregnancy. Due to these patterns of use and remaining challenges relating to reducing smoking in pregnancy, a multi-agency group comprises of some of the main health professional and research organizations, along with health charities and mother and baby organizations in England (the Smoking in Pregnancy Challenge Group) have developed practical guidance on vaping during pregnancy. This proposes a positive policy framework related to e-cig use during pregnancy for women who cannot quit smoking. Here, we will discuss the current evidence and recommendations for practice in the UK.

Lessons Learned about Prenatal E-cigarette Exposure from In Utero Exposures in Animal Models
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Recent epidemiological data indicate that the popularity of electronic cigarettes (e-cigarettes), and consequently nicotine use, is rising in both adolescent and adult populations. As nicotine is a known developmental neurotoxin, these products present a potential threat for those exposed during early life stages. Despite this, few studies have evaluated the toxicity of e-cigarettes on the developing central nervous system (CNS) or on reproductive outcomes of the impacted offspring. This presentation will focus on the effects of early life exposure to e-cig aerosols with and without nicotine on neurdopment and later life behavior, as well as alterations on male offspring reproductive function. In addition, we will discuss the effects of maternal e-cig exposure with and without nicotine on adverse obstetric outcomes including birth weight and pregnancy duration. This presentation will lay toxicological groundwork demonstrating the potential public health threat of e-cigarettes for the developing fetus and offspring.
Extensive efforts in research and development have led to extraordinary advances in nanoscience, nanotechnology, and the utility of nanomaterials. Uses of nanomaterials range from structural improvements, to building materials, to improvement in medical devices and drug delivery. As a result, there has been an increase in the potential for human exposure to nanomaterials not only inadvertently in occupational settings, but also in consumers and patients that utilize nanomaterials. Multiwalled carbon nanotubes (MWCNT) are a class of nanomaterials used for a variety of applications including structural enhancement of building materials and sports equipment, and for improvement of electrical and thermal conductivity for electronics. Animal studies have largely focused on the health effects following pulmonary exposure in rodent models. Many of these studies have demonstrated the occurrence of pulmonary inflammation associated with fibrotic changes in the airways. However, the majority of these studies investigated pulmonary exposure at dose levels that are significantly higher than occupational exposure levels.

Recent studies have also demonstrated that pulmonary exposure to carbon nanotubes can result in systemic effects (cardiovascular changes and immunotoxicity), including increased expression of inflammatory cytokines genes in splenic lymphocytes in rats. These findings suggest that carbon nanotubes could target the immune system and raise the concern that low-level exposure may impact host resistance to infections or development of neoplastic diseases or increase susceptibility to allergy or autoimmune diseases. To address this issue, a consortium of academic and federal scientists initiated an integrated approach to investigate the toxicity of low-level occupationally relevant exposures to a representative high-purity “long and thin” aggregated MWCNT. Characterization of this MWCNT shows that it aggregates into micron-sized “cotton ball” structures that morphologically appear similar to MWCNTs sampled in the workplace. Key features of the approach were the well-characterized, GLP-compliant inhalation exposure system used for treatment of the rats and the partnership between the federal and academic laboratories assessing different models of impacts of the exposure on the immune system. Importantly, all of the immunotoxicity studies that will be discussed in this session were obtained from the same exposure cohorts, thereby enhancing the ability to integrate results across all studies and participating laboratories. The session will identify the occupational exposures to MWCNT, put the experimental work into a human-relevant context, and then highlight the complementary approaches undertaken by the different groups to characterize the effects of MWCNT on innate and adaptive immune responses following low-dose pulmonary inhalation exposures.

Inhalation exposure to MWCNT has been shown to exacerbate lung inflammation in rodent models of pre-existing allergic lung disease. However, to date no studies have addressed the effect of allergen post-exposure following inhalation of occupationally relevant doses of MWCNT. Male B6C3F1/N mice were exposed by whole body inhalation for 6 hours a day, 5 days a week, for 4 weeks to 0.06, 0.2 and 0.6 mg/m³ of MWCNTs. Mice then received 25 μg of HDM by intranasal aspiration 6 times over 3 weeks. Mice were necropsied at 3 and 30 days after the final HDM dose to evaluate changes in cell differential counts and cytokines in bronchoalveolar lavage fluid (BALF), IgE in serum and lung pathology. MWCNT exposure reduced TH2 immune responses 3 days after HDM post-exposure as evidenced by reduced eosinophils and IL-5 in BALF, reduced airway mucus cell metaplasia and reduced serum IgE. However, inflammatory lesions around small airway and vessels were only observed 3 days after HDM allergen in mice exposed to MWCNTs. Moreover, pro-fibrotic cytokines (IL-1β and TGF-ß1) and airway fibrosis were increased at 30 days by MWCNT exposure in a dose-dependent manner with or without HDM allergen post-exposure. In conclusion, inhaled MWCNTs reduced TH2 pulmonary immune responses to allergen in mice but caused pro-fibrotic events regardless of allergen post-exposure.
BHV-0223 is a novel 40 mg rapidly sublingually disintegrating (Zydex™) formulation of riluzole, bioequivalent to conventional riluzole 50 mg oral tablets, that offers a lower-dose treatment option for ALS and potentially less risk of liver toxicity. In patients on oral riluzole, approximately 50% experience alanine transaminase (ALT) levels above upper limit of normal (ULN), 8% above 3x ULN, and 2% above 5x ULN. To quantitatively and mechanistically compare the liver toxicity potential of oral riluzole versus BHV-0223, combining clinical and mechanistic in vitro data, using DILIsym®. DILIsym® (DILIsym Services, Research Triangle Park, NC) is a validated multi-scale computational model that supports evaluation of liver toxicity risks. BHV-0223 (40 mg BID) and oral riluzole (50 mg BID) were simulated by combining physiologically based pharmacokinetic modeling with mechanistic liver toxicity parameters derived from in vitro assays. Frequencies of ALT elevations were predicted in the simulated populations (SimPops). In the SimPops analysis, ALT >3x ULN was observed in 3.9% (1/285) versus 1.4% (4/285) of individuals with oral riluzole and sublingual BHV-0223, respectively. The validity of the DILIsym® representation of riluzole and assumptions is supported by its ability to predict rates of ALT elevations for oral riluzole comparable to that observed in clinical data. Combining a mechanistic, quantitative representation of hepatotoxicity with inter-individual variability in both susceptibility and liver exposure suggests that sublingual BHV-0223 confers diminished rates of liver toxicity compared to oral tablets of riluzole, consistent with having a lower overall dose of riluzole and bypassing first-pass liver metabolism.

With the surge of technology and continuing improvement of assays for better testing, scientists are increasingly in a position to ask whether or not to adopt new technologies. The lure of increased sensitivity and specificity, often accompanied by large price tags, put into conflict the scientists’ responsibility to contain costs and their obligation to make use of the best tools available. In this work, we present a visualization tool to help scientists swiftly evaluate the worth of new assays and means to improve the speed and quality of their decisions on whether or not to adopt them. Since each parameter (x) relevant to use of a toxicity test (prevalence, sensitivity and specificity) has a value between 0 and 1, the intervals [0, x] and [x, 1] define a partition on [0, 1]. The proper arrangement of these partitions can be used to define areas in [0, 1] x [0, 1] cross-space that partitions the cross-space into a set of areas. Our analogy is a square plot of land subdivided into smaller lots. We call the resultant graphic a real estate diagram. As an example, the experience in predicting human clinical Thorough QT (TQT) studies was recently described (Park et al., 2013). The data for a total of 204 compounds could be divided into chronological sets: we illustrate 2 blocks of two years 2005–2006 and 2011–2012. In the first block 13 of 29 submitted compounds had positive TQT studies; in the second block the prevalence was 4 of 42. In another study (Park et al., 2018) the hERG channel patch-clamp assay used in predicting TQT outcome had a sensitivity of 0.64 and specificity of 0.72. The test was considered positive if the inhibitory concentration for 50% block (IC50) was within 100x the clinical test concentration. Real estate diagrams were constructed to yield insight into the PPV and NPV of the TQT prediction. The structure of the real estate diagrams clearly illustrates that as prevalence has fallen increasing assay sensitivity will have a trivial effect on PPV and NPV. In the NPV case, because of the very low prevalence, it would be better to base decisions on NPV and cease expending resources on improving the assay’s sensitivity or specificity. As the scientists managing toxicity testing, toxicologists will be called upon to question whether their safety assessments regarding specific toxicities should be based on their assay’s positive or negative predictive value, the real estate diagram is a useful tool in making that assessment. References: Park, E., et al., (2013). International Journal of Cardiology, 168(5), 4975–4976. Park, E., et al. (2018). British Journal of Pharmacology, 175(4), 606–617.

The 2015 US FDA’s guidance for industry provides permissible daily intake (PDE) limits for elemental impurities through oral, parenteral and inhalation routes of exposure in human drug products but not for dermal or other dosing routes. The risk evaluation for elemental impurities via the transdermal delivery routes have not been explored. This evaluation focused on systematic bioavailability of 9 metal (As, Cd, Pb, Hg, Co, Ni, V, Pt and Pt) impurities. In each case, a transdermal PDE was extrapolated from the oral PDEs derived in 2015 US FDA’s Q3D Elemental Impurities Guidance for Industry (https://www.fda.gov/downloads/drugs/guidances/ucm371025.pdf). The transdermal bioavailability values derived are based upon a systematic review and evaluation of primary and secondary sources of literature. In considering transdermal PDE determination, we assumed similar systemic toxicity as from the oral route. We focused on the bioavailability of the metals as the potential toxicity to systemic targets. Estimates of metal bioavailability on skin ranged from 0.1 to 5% with a useful default assumption of 0.1% dermal absorption, if presented as dry particulate (dust) or 1% for liquid medium. However, some of the reviewed metals have exhibited toxicity on the skin or dermal hypersensitivity reactions which have merited consideration in setting dermal PDEs. This approach is compatible with Q3D guidance Option 3: Finished Product Analysis, in which the finished product (rather than individual components) can be evaluated against a PDE, which considers the bioavailability of the impurity within the product. The results of this risk evaluation yielded the transdermal PDEs involving pharmacokinetic adjustment of the oral PDE provided by US FDA 2015. The ratio of the oral to dermal bioavailability was calculated and multiplied by the oral PDE to derive the dermal for each element. Our analysis calculated the transdermal PDEs (ug/day) for each elemental impurity were: As (15), Cd (50), Pb (500), Hg (300), Co (50), Ni (200), V (1000), Pt (100) and Pt (100). PDE adjustment is also considered with respect to whether the elemental impurity is known to produce direct toxicity or hypersensitivity to the skin, in which case that becomes a key consideration in developing a dermal PDE for that impurity. Disclaimer: Authors declare that views expressed are of their own and have no conflict of interest of any nature or kind.

Off-target Activities of Troglitazone and Rosiglitazone in Tox21/ToxCast In Vitro Tests and Comparison of the Results with Systematically Reviewed In Vivo Animal Data and Human Adverse Events from Pharmacovigilance Databases Using Evidence-Based Approaches

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In order to study the ability of in vitro data to predict the hazards of chemicals, we analyzed the in vitro data from US EPA ToxCast™ (ToxCast) program of Troglitazone and Rosiglitazone, and compared it with the profile obtained from a systematic literature review on the results of standard toxicity tests in experimental animals and findings in adverse events (pharmacovigilance) database. Both Troglitazone and Rosiglitazone are considered as drugs: Troglitazone was withdrawn from the market because of drug-induced liver injury (DILI), while Rosiglitazone was linked to less DILI concern. Biological off-target activity analysis using ToxCast in vitro tests, normalized to the human C50 values, showed that Troglitazone had biological off-target activity in 50% more assays compared to Rosiglitazone. A protocol-driven systematic literature review of liver-associated effects of the 2 drugs in dogs, mice, rats, non-human primates and randomized human clinical trials was performed to serve as a comparator. In total after reviewing 6,805 studies, 105 abstracts for Troglitazone and 175 studies for Rosiglitazone were included for full-text review, following which the data was extracted from the included papers using a standardized form. A profile of adverse liver effects of the 2 drugs in humans and animal models will be presented. To incorporate the real-world data on frequencies of adverse effects in patients, the WHO Vigibase pharmacovigilance dataset was analyzed for DILIs events upon treatment with
the 2 drugs and a profile of reported MedDRA terms will be presented. More liver-specific adverse effects were recorded for Trogilitazone compared to Rosiglitazone. Results of these 3 data streams will be presented. Challenges with using evidence-based methods in this project and some solutions that the EBTC workgroup has found will be described.

2612 Supportive Care for Animals on Toxicology Studies: An Institutional Benchmark Survey


Abbvie, North Chicago, IL; Janssen, Spring House, PA; Bristol-Myers Squibb Co, New Brunswick, NJ; Covance, Madison, WI; Charles River Laboratories, Wilmington, MA; Boehringer-Ingelheim, Ridgefield, CT; and Amgen Research, South San Francisco, CA.

Contract Research Organizations (CROs) conducting toxicity studies on behalf of pharmaceutical sponsors routinely provide supportive care for animals on study. There is currently limited industry guidance on the definition and strategies for administering supportive care in toxicity studies. The 3Rs Leadership Group of the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ Consortium) created a CRO Outreach Working Group (WG) at the beginning of 2015. One of the 2018 goals of this WG was to conduct a survey to better understand the scope of the information that is shared between pharmaceutical sponsors and CRO partners with regards to the decision process about the provision of supportive care. The survey also aimed to define supportive care strategies, identify alternative options and understand regulatory requirements for supportive care. The survey was distributed to the 3Rs Leadership Group of the IQ Consortium, and to several CRO partners, representing 35 organizations as potential respondents. Feedback on the survey questions was collected and analyzed to benchmark current practices and a potential path forward. The results of the survey form 13 respondents indicated the need to provide adequate information at the drafting stage of study design and several potential methods for future communication improvement between the pharmaceutical sponsors and CROs. Areas of enhancements were identified including greater consistency in the inclusion of sponsor veterinarians on project teams for externalized studies, the timing of initiation of supportive care, and a lack of sharing of regulatory feedback outcomes. Suggested best practices include having CRO veterinary staff involved in initial study planning meetings, creating a plan of action for veterinary care prior to study start, enhancing information sharing regarding expected toxicities from previous study findings, and sharing CRO guidelines on humane endpoints and animal welfare standards. Improved communication regarding supportive care will pave the way for enhanced 3Rs initiatives, refining the existing animal care paradigm and helping to ensure the most ethical toxicity study designs.

2613 New Opportunities for a Psychoactive Psychilysin in Neuropsychopharmacological Clinical Disorders

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Abnormalities of neuropsychopharmacological function of the Central Nervous System (CNS) can result in morbidity and disease that have a huge impact on society as well as the wellbeing of the individual. As the CNS plays a central role not just in the field of psychiatry but also in the physiological control of many bodily functions, such disorders have the capacity to be manifest widely across different fields of medicine. Basic and clinical research into neuropsychopharmacological disease is ongoing within academia and the pharmaceutical industry but there is still an unmet medical need in many areas. What is becoming increasingly clear is that some previously researched pharmacology and drugs may present opportunities yet to be realised. The psychoactive drug psilocybin is currently under clinical development for potential benefit in treatment resistant depression. The compound, originally derived from a range of mushrooms including Psilocybe species, has been used recreationally and spiritually for hundreds of years. Additionally, there have been limited clinical studies in the psychiatric field in the 1960s. Although humans have been exposed to psilocybin over many decades, either illicitly or as an active agent in many clinical trials, no formal good laboratory practice (GLP) safety studies have ever been conducted under its safety. To supplement existing published information on the effects of psilocybin in animals and humans, we performed to GLP a hERG assay, a battery of genetic toxicity studies and an extended single dose study in rats at maximum tolerated dose (MTD). Unlike the development of a new chemical entity, the purpose was not to produce a dose response curve and then delineate a “safe” clinical dose. Rather, it was to confirm, under GLP, that we understood the toxicology in relation to previously published non-GLP data from experiments and clinical trials and to establish that there were no unreported toxicities that needed to be taken into consideration. Psilocybin was shown to have low potency interaction with the hERG channel (minimal effect at 1000µM and no effect at 100µM at a membrane potential of -60mV). It was also shown to produce a range of effects in rats that were entirely consistent with previously reported effects in animals and humans. This toxicology package filled significant gaps in the existing information in the literature and supported progression to clinical trials in treatment resistant depression.

2614 Functional, Histologic and Ultrastructural Assessment of Novel Optical Coherence Tomography Findings in the Cynomolgus Monkey


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During development of four novel intravitreal therapeutics in the Cynomolgus monkey, optical coherence tomography (OCT) in the 24hr post-dose period identified two novel OCT findings not previously observed or reported in association with intravitreal (IVT) dosing. The first finding was a sub-foveal hyper-reflective band (HRB) in the space between the EZ line and RPE. The second was a hyper-reflective line at the junction of the photoreceptor outer segments (POS) and the retinal pigment epithelium (RPE), either isolated or peripheral to areas of retinal detachment, and not limited to the fovea. To further characterize these OCT findings and better understand the potential for translation and human risk, a study was undertaken in the non-human primate. Eight Cynomolgus monkeys were dosed bilaterally via ITV injection with a 100µl high osmolality vehicle previously identified as producing these OCT findings; or pegaptanib sodium, a clinically well-tolerated, large volume IVT injection. Following dosing, OCT was conducted to establish the presence and character of any changes in OCT from baseline, followed immediately by multi-focal electrophysiology (mERG), to help understand the functional consequence of these OCT findings. The animals were then euthanized, perfusion fixed and the eyes were processed for ultrastructural examination. OCT findings of HRB and HFL lines were observed in 5/8 eyes treated with high osmolality vehicle and 1/8 eyes treated with pegaptanib sodium. HFL were observed in 6/8 eyes treated with high osmolality vehicle, but were not identified in animals administered pegaptanib sodium. Functional consequences, manifest as changes in mERG, were not observed. Ultrastructurally, the HFL line was correlated with expansion of the photoreceptor layer, with increased space between the photoreceptor outer segments (consistent with edema). The HRB observed was consistent with regions of disk separation and distorion at the junction of the inner and outer photoreceptor segments. Ultrastructural correlates of both HRBs and HFLs were considered consistent with a transient, reversible lesion, in line with the rapid resolution of these findings on OCT. Because of the histopathological and ultrastructural nature of these lesions, and the lack of functional correlates, these novel OCT findings are considered to be non-adverse.

2615 Intestinal Toxicity in Rats Following Administration of CDK4/6 Inhibitors is Independent of Pharmacology


1Pfizer, San Diego, CA; 2Pfizer, Groton, CT; and 3Pfizer, Pearl River, NY.

Recently three different cyclin-dependent kinase 4 and 6 (CDK4/6) dual inhibitors were approved for the treatment of breast cancer (palbociclib, ribociclib and abemaciclib), all of which offer comparable therapeutic benefits. Their safety profiles however are different. For example, neutropenia is observed at 4 times higher dose for palbociclib compared to the other drugs, novel in that it is the most common adverse event for palbociclib and ribociclib, whereas diarrhea is the most common adverse event observed in patients treated with abemaciclib. In order to understand the mechanism of diarrhea associated with this class of drugs and to establish that there were no unreported toxicities that needed to be taken into consideration, Psilocybin was shown to have low potency interaction with the hERG channel (minimal effect at 1000µM and no effect at 100µM at a membrane potential of -60mV). It was also shown to produce a range of effects in rats that were entirely consistent with previously reported effects in animals and humans. This toxicology package filled significant gaps in the existing information in the literature and supported progression to clinical trials in treatment resistant depression.
2616 Assessing Immunogenicity of Host Cell Proteins in BLT-Immune Humanized Mice
S. B. Hosain, Y. Yan, C. L. San Emeterio, A. D. Knapton, and K. E. Howard.
US FDA, Silver Spring, MD. Sponsor: J. Weaver.

In recent years, development and clinical use of biologics has increased significantly due to their ability to effectively treat immune-mediated diseases and cancer. However, immunogenicity to biologics remains a major concern as repetitive administration of biologics can trigger immune responses in the form of anti-drug antibodies (ADAs). One potential cause of immunogenicity is host cell proteins (HCP). HCP represent cellular proteins from non-human cell lines that occur through the manufacturing process. Despite extensive purification and filtration processes, residual levels of HCP are present in marketed products. It is unclear if these proteins can serve as adjuvants to the immune system and help induce formation of ADA. Therefore, we tested various unique HCP fractions produced at US FDA in a bioassay using standard antibody production cell lines in bone marrow-liver-thymus (BLT) immune humanized mice to determine their ability to induce measurable immune responses in the absence of active pharmaceutical product. Groups of four–eight BLT-immune humanized mice were treated subcutaneously either 10 ng or 100 ng of one of five preparations of HCP or saline three days per week for eight weeks. As a positive control, one group was treated with hepatitis A vaccine. We assessed immune activation via flow cytometry in serial blood samples through changes in T cell activation markers. Cytokines and antibody production were evaluated through periodic serum sampling. Results showed that measurable increases in activation markers on T cells were evident in HCP-treated mice. We observed increased levels of plasma cells in bone marrow with specific preparations of HCP, and administering greater amounts of HCP did not necessarily cause more immunogenicity. Significant increases in T cell populations were observed with HCP treated mice but not saline controls. We also assessed immunoglobulin class switching and found that some HCP-treated groups developed IgG2 or IgG4 antibodies, suggesting maturation of antibody responses and cross talk between B and T cells in HCP-treated mice. Our results show that unique responses can occur to preparations of HCP and that repeated exposure to HCP alone can induce an immunogenic response in human immune cells.

2617 Safety Testing of Challenge Agents
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Challenge agents (molecules that induce a response that can be inhibited by the therapeutic) may be used in clinical trials to support the pharmacodynamic (PD) activity of the therapeutic being tested. In general, these agents have been in limited human subjects but the nonclinical safety regulatory requirements for use in clinical trials are unclear. A target-specific peptide, maxadilan, a vasodilator, was utilized to demonstrate biological activity of a PAC1 (pituitary adenylate cyclase receptor-type 1) antagonist mAb measured by a dermal blood flow assay (DBF) using laser Doppler. Maxadilan administered intradermally was shown to increase DBF in the rat and cynomolgus monkey; this response was blocked by an anti-PAC1 antagonist mAb demonstrating utility as a challenge agent. To support the PD clinical trial, a 2-week intradermal (ID) and intravenous (IV) toxicology study was conducted in the rat. Rats were administered 0.3 to 30 μg maxadilan ID on Days 1, 7, and 14 or 0.005 μg/kg IV on Day 14. Clinical observations included red skin (pinnae) at >0.3 μg ID, or >0.005 μg/kg IV and salivation (both ID and IV high dose groups). Clinical pathology changes were consistent with mild indications of inflammation (increased WBCs and fibrinogen). Histologic myocardial degeneration was observed at >0.3 μg ID, and coronary artery necrosis (n=1 of 5) was present at 30 μg ID. These cardiovascular light microscopic changes were consistent with anticipated pharmacology of maxadilan. A second ID challenge agent, PACAP, was also planned to be used in a PD clinical trial. To support the PACAP challenge agent, an extended single dose rat IV study, and a rat safety pharmacology study were completed. Similar clinical findings to maxadilan were observed in the rat following doses of 0.005, 0.05 or 0.5 mg/kg in addition to increased fibrinogen and myocardial fibrosis. In the safety pharmacology study, a transient increase, then decrease in heart rate was observed in addition to a transient decrease in mean arterial pressure followed by an increase in pulse pressure were observed at all doses. A decrease in body temperature was seen at the mid and high dose. In summary, these nonclinical studies supported Phase 1 clinical trials using maxadilan and PACAP as challenge agents.

2618 The Protective Effect of Rosiglitazone against Electronic Cigarette/Tobacco Smoke-Induced Cerebrovascular Toxicity
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The content of tobacco smoking (TS) and also e-cigarettes (EC) are associated with vascular endothelial dysfunction in a causative and dose-dependent manner primarily related to the content of reactive oxygen species (ROS), nicotine, oxidative stress, and smoking-induced inflammation. It is well established that both TS and EC promote glucose intolerance and increases the risk of developing type-2 diabetes mellitus (2DM) with which it shares other pathogenic traits including the high risk of neurological and cerebrovascular disorders via ROS generation, blood-brain barrier (BBB) impairment and inflammation. Herein, we investigated the protective effect of rosiglitazone (RSG) against oxidative stress damage at the BBB by chronic TS/EC exposure. For this purpose, we assessed intracerebral ROS generation and BBB integrity using immunofluorescence, western blotting, Intracellular ROS generation, and TEER measurement, as well as BBB permeability analyses using labeled dextran in mouse brain. Experiments were conducted on TS/EC-exposed mouse brain microvascular endothelial cells (mBMVEC-P3) treated with RSG (20 μM) based on cell viability evaluated by MTT cytotoxicity. Results were compared to corresponding untreated TC/EC exposed cultures. The results revealed that RSG as a peroxisome proliferator-activated receptor gamma (PPARY), at pharmacologically relevant levels, inhibited the NOD2 and PPARy pathway which reduced ROS generation and TS/EC toxicity at the cerebrovascular level. The results also confirm the RSG’s role in the reduction of inflammation and oxidative stress and also suppression of tight junction (TJ) protein downregulation and loss of BBB integrity induced by TS/EC. In contrast, nimesulide was kept for additional 3 months without further MH treatment. In MD mice, the typical TN symptoms were induced as expected, reflected by increased protein urine, renal lipid accumulation and lipotoxic effects inducing oxidative stress, and inflammatory reactions, and final fibrosis. However, these typical TN changes were significantly prevented by MH treatment at time points of 3 months after 3-month MH treatment, suggesting MH-induced renal protection from T2D has memory effect. Mechanistically MH renal proximal tubules from TN may be related to its lipid metabolic improvement by the activation of AMPK/PGC-1a/CPT1-mediated fatty acid oxidation. In addition, MH regulates glucose metabolism through the mTOR-AKT pathway. These results indicate that treatment of T2D with MH effectively prevents DN probably via AMPK-dependent improvement of glucose and lipid metabolisms. However how MH-activated AMPK pathways can persist long time even 3 months after completing 3-months of MH treatment remains further study.

2619 4-O-Methylhonokiol Ameliorates Type 2 Diabetes-Induced Nephropathy in Mice by Inhibiting Inflammation and Oxidative Stress
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Diabetic nephropathy (DN), characterized by proteinuria and accumulation of extracellular matrix (ECM) proteins, is one of the most serious long-term complications of type 2 diabetes (T2D). 4-O-methylhonokiol (MH) is considered one of the major bioactive constituents of Magnolia stem bark. In our previous studies, we observed that MH partially attenuated heart damage in a HFD-induced obese mouse model. In this study, we aim to elucidate whether treatment with MH can ameliorate or slow-down progression of DN in a T2D murine model and, if so, whether the protective response of MH is associated with AMPK-associated anti-oxidant and anti-inflammatory effects. Mice were fed normal diet or high fat diet for 3 months to induce insulin resistance and then intraperitoneal injection of STZ to induce hyperglycemia as T2D model. Both T2D and control mice received gavage containing vehicle or MH once diabetes onsets for three months. Five mice from each group were sacrificed once completing 3-month MH treatment. The remaining anesthetized mice were kept for additional 3 months without further MH treatment. In T2D mice, the typical DN symptoms were induced as expected, reflected by increased proteinuria, renal lipid accumulation and lipotoxic effects inducing oxidative stress, and inflammatory reactions, and final fibrosis. However, these typical TN changes were significantly prevented by MH treatment at time points of 3 months after 3-month MH treatment, suggesting MH-induced renal protection from T2D has memory effect. Mechanistically, MH renal protection from DN may be related to its lipid metabolic improvement by the activation of AMPK/PGC-1α/CPT1-mediated fatty acid oxidation. In addition, MH regulates glucose metabolism through the mTOR-AKT pathway. These results indicate that treatment of T2D with MH effectively prevents DN probably via AMPK-dependent improvement of glucose and lipid metabolisms. However how MH-activated AMPK pathways can persist long time even 3 months after completing 3-months of MH treatment remains further study.
2620 Reactive Oxygen Species Produced by the Corrosion of Fe-Based Materials Induce Endothelial Dysfunction.

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Fe-based materials are currently considered for manufacturing biodegradable coronary stents. Here, for the first time, we document the generation of reactive oxygen species (ROS) during Fe-based materials corrosion and their deleterious impacts on endothelial function. ROS generation was documented by the acellular terephthalic acid (TA) hydroxylation assay. Thoracic rat aortic rings were isolated from Wistar rats and endothelium was directly exposed (6 h) by inserting an iron wire into the lumen in the presence/absence of catalase, an antioxidant enzyme. Indirect exposure was included as a control to assess the potential role of solubilized iron ions by incising aortic rings with an iron wire outside the lumen. Nitric oxide (NO) production by aortic rings was measured by EPR spin-trapping, and endothelium-dependent relaxation in response to muscarinic stimulation was assessed with the organ-bath method (doi: 10.1371/journal.pone.0152579). Induction of oxidative stress in aortic rings was assessed via the mRNA expression of the oxidative stress response gene heme oxygenase-1 (HO-1). The permanent material currently used for coronary stents, the austenitic stainless steels 316L, was used as negative control in all tests. Iron exhibited a strong potential to generate ROS in the TA assay. ROS generation, but not corrosion, was inhibited by catalase. Direct contact with iron significantly impaired endothelium-dependent relaxation of aortic rings. Direct contact with the material in the presence of catalase, or indirect contact did not affect endothelium function. NO production by treated rat thoracic aortic rings was inhibited by ROS generated by the corrosion of iron, as indicated by the protective role of catalase. Expression of HO-1 was increased after direct exposure to iron, and not increased to indirect exposure or in the presence of catalase. The demonstration of ROS production during corrosion, induction of oxidative stress, inhibition of NO production and consequent endothelium dysfunction raise concern about the biocompatibility of biodegradable Fe-based alloys for vascular implants.

2621 Polyethylene Glycol (PEG) Coated Liposomes Increase Oxidative Stress in Endothelial Cells

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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. We have previously shown that PEG-coated liposomes attenuate endothelium-dependent arterial dilation, but the mechanism(s) of action is unknown. Preliminary data indicates that changes in reactive oxygen species (ROS) generation may play a role in the observed microvascular impairments; therefore, we hypothesize that PEG-coated liposomes will increase oxidative stress and inflammation in endothelial cells thereby contributing to microvascular dysfunction. Rat aortic endothelial cells were treated with PEG or non-PEG coated liposomes (3, 25, and 100 µg/ml). Flow cytometry was used to determine changes in ROS. Vascular activation [intercellular adhesion molecule-1 (ICAM)] and vascular cell adhesion molecule-1 (VCAM)), inflammation [interleukin (IL)-6], and anti-oxidant (Heme oxygnase-1, HemOx) gene expression changes were assessed via PCR. Additionally, oxidative stress potential was assessed by analyzing the ratio of glutathione disulfide (GSSG) to glutathione (GSH) via high performance liquid chromatography (HPLC). There was a significant increase in ROS following exposure to PEG-coated liposomes compared to the non-PEG liposomes and vehicle control (2.58 ± 0.56 vs. 1.39 ± 0.13 and 1 ± 0.15-fold change, respectively). There was also an increase in oxidative stress potential in the endothelial cells exposed to the highest dose of PEG and non-PEG coated liposomes (16 ±4 and -10.5 ± 4, respectively) as determined by an increase in the GSSG/GSH ratio. Finally, there was a 1.5-fold increase in VCAM and 3.5 and 7-fold increase in HemOx gene expression compared to control following treatment with 25 and 100 µg/ml of PEG-coated liposomes, indicating potential vascular activation and anti-oxidant alterations. Taken together, these results indicate liposomes may alter oxidative stress and vascular activation in endothelial cells, which may contribute to the previously observed microvascular impairments. It is critical to understand liposome formulations, including coatings, on both endothelial and vascular response for their safe implementation in medicine.

2622 3D Hepatocyte Spheroid Models in High-Throughput Transcriptomics and Benchmark Concentration Analysis for Predictive Liver Toxicology Screening


Improved tissue-like functionality with three-dimensional (3D) spheroid models offer better in vitro safety assessment of chemicals. Combining these models with novel low-cost, high-throughput transcriptomics methods would enable systematic characterization of chemical induced effects on biological systems. We have developed 3D hepatocyte spheroid models of human hepatic progenitor cell line, HepaRG and primary rat hepatocytes (Sprague-Dawley) in 384 well formats. HepaRG spheroids exhibit several hallmarks of polarized hepatocytes and tissue-like functionality. Xenobiotic metabolism assessment with clinical substrates of CYP1A2, CYP2B6 and CYP3A4 showed 2 to 20-fold higher than median activities compared to two-dimensional (2D) cultures of primary human hepatocytes (PHH). Similarly, primary rat hepatocytes also formed polarized spheroid-like structures and displayed a stable phenotype for more than 30 days compared to 2D sandwich cultures which are healthy for 3-5 days in culture. Specific activities of CYP1A1 and CYP3A enzymes were markedly higher than sandwich cultured rat hepatocytes. HepaRG spheroids were treated with ten concentrations of a reference set chemicals for 96-hours in repeated exposure regimens and assays using high-throughput gene expression analysis with the S1500+ gene set were performed. Baseline gene expression and biological pathway responses showed a significantly higher enrichment score in spheroids compared to 2D HepaRG cells. Exposure to aflatoxin B1 and benzo(a)pyrene showed activation of genes and pathways related to cell cycle, DNA damage and cancer at low non-cytotoxic concentrations. BMDExpress 2.2 was used to calculate point of departure for individual genes and pathways associated with concentration-related molecular perturbations. Activation of nuclear receptor pathways (CAR/RXR and PXR/RXR) was evident with lower exposure levels of phenobarbital and rifampicin preceding onset of stress-related pathways at higher cytotoxic concentrations. Similarly, biologically relevant pathways associated with exposure of 4-(methyltrinitroamino)-1-(3-pyridyl)-1-butane (NINK), cyclophosphamide, chlorpromazine and valproic acid were activated at sub-lethal concentrations in HepaRG spheroids. Incorporation of these physiologically relevant in vitro models and data-rich assay approaches are promising new tools in identifying sensitive molecular perturbations that lead to adverse effects of chemicals.
A Human In Vitro Skin Explant Assay to Assess the Immunotoxicity of Biopharmaceuticals and Aggregated Monoclonal Antibodies


To aid preclinical prediction of adverse immune reactions to biopharmaceutical drugs, we have developed a human in vitro skin explant assay. This assay uses autologous human blood and skin to assess histopathological damage caused by adverse immune reactions. This assay is a valuable preclinical tool for safety assessment of biologics. We tested 17 commercial biologics in the skin explant assay (n=10) and correlated the results with clinical occurrence of adverse hypersensitivity reactions. Results showed a statistically significant positive correlation between adverse clinical outcome and response in the skin explant assay (r=0.815, p<0.0001). Drugs known to cause adverse reactions e.g. Campath® and OKT3® gave a positive skin histopathology Grade II or above in the (34-90)% of individuals, respectively. An analogue of the TGN drug, TGN1412, gave a Grade III response in 90% of donors tested. Drugs which have been defined as clinically negative for adverse immune reactions, such as Enbrel® and Cimzia®, showed a negative response, with 10% and 30% of individuals reacting with the drug, respectively. We also assessed adverse reactions to biosimilars (Remsima® and Inflectra®) against the reference product (Remicade®). Overall, a negative response was observed for all three biologics in the skin explant assay. Interestingly, drug responses varied among donors, indicating that the assay may be able to predict the best personalised therapy for an individual patient. The assay was also used to assess if aggregated monoclonal antibodies gave rise to adverse immune reactions. Monoclonal antibodies were aggregated by heat (40°C) protocol and characterised by adverse hypersensitivity reactions. Transmission electron microscopy, revealing aggregation levels ranging from 2.5-6.0% of total protein content. Results showed that aggregated Herceptin® gave a Grade II positive response in 80% of donors and aggregated Rituximab® gave a Grade III positive response in 60% of donors tested. Sections also stained positive for Heat Shock Protein 70 (HSP70) as a marker of aptosis. Our novel human in vitro assay showed that it was highly sensitive for determining adverse immune reactions to biologics and aggregated mAbs and is a promising tool for the prediction of immunotoxicity.

Metallothionein Improves Angiogenic Function of Endothelial Progenitor Cells via HIF-1α/SDF-1/Akt Pathway in Diabetic Ischemia

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Diabetes mellitus associated dysfunction of endothelial progenitor cells (EPCs) may contribute to dysregulation of endothelial regeneration. Metallothionein (MT) acts as an antioxidant in various pathophysiological processes. Whether MT in EPCs has positive angiogenic effects and the mechanisms involved remain unclear. Endothelial MT overexpression (JTMT) mice were made diabetic by high fat diet followed by STZ administration (HFD/STZ). Diabetic and control hind limb ischemia model was established by femoral artery ligation. Bone marrow derived mononuclear leucocytes (MNCs) collected from JTMT mice were transplanted into db/db mice with hind limb ischemia. Blood reperfusion was monitored by a microcirculation imaging system and angiogenesis of ischemic muscle was assessed by CD31 staining. Oxidative stress was measured by DHE staining and western blot of 3-NT and 4-HNE. High glucose and hypoxia conditions in culture were adopted to mimic diabetic ischemia for mechanistic studies in EPCs. The critical role of HIF-1α in MT protection of EPC function was explored by siRNA knockdown of HIF-1α. MT improved blood perfusion of ischemic hind limb and promoted angiogenesis of ischemic muscle. MT overexpression of JTMT mice was significantly higher than control mice, which was highly correlated with mitochondrial respiration. Bone marrow derived mononuclear leucocytes (MNCs) collected from JTMT mice were transplanted into db/db mice with hind limb ischemia. Blood reperfusion was monitored by a microcirculation imaging system and angiogenesis of ischemic muscle was assessed by CD31 staining. Oxidative stress was measured by DHE staining and western blot of 3-NT and 4-HNE. High glucose and hypoxia conditions in culture were adopted to mimic diabetic ischemia for mechanistic studies in EPCs. The critical role of HIF-1α in MT protection of EPC function was explored by siRNA knockdown of HIF-1α. MT improved blood perfusion of ischemic hind limb and promoted angiogenesis of ischemic muscle. MT overexpression of JTMT mice was significantly higher than control mice, which was highly correlated with mitochondrial respiration. The expression of HIF-1α, SDF-1, VEGF and Akt signaling were all increased in JTMT mice, along with elevation of plasma SDF-1α and VEGF. Compared with WT mice, the expression of HIF-1α, SDF-1, VEGF and Akt signaling were all increased in JTMT mice, along with elevation of plasma SDF-1α and VEGF. Compared with WT mice, the expression of HIF-1α, SDF-1, VEGF and Akt signaling were all increased in JTMT mice, along with elevation of plasma SDF-1α and VEGF. Compared with WT mice, the expression of HIF-1α, SDF-1, VEGF and Akt signaling were all increased in JTMT mice, along with elevation of plasma SDF-1α and VEGF. Compared with WT mice, the expression of HIF-1α, SDF-1, VEGF and Akt signaling were all increased in JTMT mice, along with elevation of plasma SDF-1α and VEGF. Compared with WT mice, the expression of HIF-1α, SDF-1, VEGF and Akt signaling were all increased in JTMT mice, along with elevation of plasma SDF-1α and VEGF. Compa...

Crosstalk between GATA4 and Mitochondria during Cardiomyocyte Differentiation upon Exposure to Tyrosine Kinase Inhibitors

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Maternal drug exposure during pregnancy increases the risks of congenital heart defects (CHDs). For instance, tyrosine kinase inhibitors (TKIs) are known to induce cardiotoxicity and mitochondrial dysfunction; they also can cross the placenta, posing high risks for CHDs. However, there are still large knowledge gaps in the underlying mechanisms underlying early-onset heart defects due to maternal drug exposure. Recent advances have improved our ability to generate cardiomyocytes (CMs) from human stem cells, which provide a great opportunity for advancing mechanistic and predictive developmental toxicology. In this study, we used human embryonic stem cells (h-iPSCs) to study interactions between CM differentiation and CM mitochondrial dysfunction. The transplantation of CMs from the iPHRECs treated with several TKIs drugs (maximun, sunitinib, and vandetanib) at sublethal levels (lower than their clinical levels) during CM differentiation. We found that developmental exposure to TKIs induced impairment of mitochondrial respiration, disarrangement of sarcomere structure, increased calcineurin levels, and significant alterations in contractility and Ca2+-handling properties of differentiating CMs. Computational biological analyses of both transcriptomic and chromatin accessibility landscape revealed that TKI-exposure altered GATA4-mediated regulatory network, which was highly correlated with mitochondrial respiration. Using a gain-of-function approach with CRISPR-activation, we observed that increases in GATA4 overexpression restored CM functions and mitochondrial respiration despite of TKI exposure. Altogether, we applied a stem-cell-based system to identify a novel crosstalk between GATA4 activity and mitochondrial respiration during CM differentiation. This study provides new insights into the relationship between gene-regulation and mitochondrial functions, and significantly enriches our understanding of sublethal TKI-induced cardiotoxicity during cardiac development.

Identification of Novel Cardioprotective Mechanisms in Doxorubicin-Induced Cardiac Injury

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Anthracycline cardiotoxicity is an established severe complication from chemotherapy. Since their discovery over 50 decades ago, the cardiotoxic effect of anthracyclines remain a significant medical problem. This study aimed to identify early cardiac changes after doxorubicin treatment and investigate the modulation of c-Myc transcriptional activity as a potential cardioprotecti...
2628 Functional Characterization of a Novel Long Non-Coding RNA Linc00844 in Drug Metabolism and Hepatotoxicity

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Long noncoding RNAs (IncRNAs) are involved in various biological and pathological processes, such as development and carcinogenesis, by regulating gene expression; however, little is known about their functions in xenobiotic metabolism. Using acetalaminophen (APAP) metabolism in hepatocytes as a model, we identified IncRNAs that were potentially associated with drug metabolism and toxicity. We examined the expression profiles of IncRNAs, key drug metabolizing enzymes (DMEs) and nuclear receptors (NRs) in APAP-treated HepaRG cells using high-throughput RNA sequencing and in silico analyses. Our results showed that Linc00844 was significantly correlated with CYP3A4, CYP2E1, SULT2A1, NR112, and HNF4A on the expression level. We found that Linc00844 was specifically expressed in the brain, liver, and kidney using the GTEx RNA-seq dataset. We also validated the expression and localization of Linc00844 via RT-qPCR and characterized its sub-cellular localization via RNA fluorescence in situ hybridization. Moreover, we performed gain- and loss-of-function assays to evaluate the role of Linc00844 on gene expression of CYP3A4, CYP2E1, SULT2A1, NR112, and HNF4A in human liver cells. Using a series of depletion experiments of Linc00844, we demonstrated that the nucleotide sequence 115-554 was essential to the function of Linc00844. Our results suggest that Linc00844 may play an important role in drug metabolism by regulating DME and NR expression.

2629 Consequences of Neonatal Activation of PXR on Drug Metabolism in Adult Mice

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Pregnane X receptor (PXR), which can be activated by xenobiotic chemicals including paediatric drugs, plays a key role in the regulation of drug metabolism enzymes (DMEs). The induction of DMEs due to the activation of PXR may reduce therapeutic efficacy or cause toxicity. The goal of this work was to demonstrate the impacts of neonatal PXR agonist exposure on DMES expression and drug sensitivity in adulthood. PCN (pregnenolone-16α-carbonitrile) was used as a PXR agonist for drug exposure at early age, C57BL/6 mice as animal model, and mice primary hepatocytes for drug sensitivity study. Both PCN dose (0, 50, 100, 150, 200 mg·kg\(^{-1}\)) and female mice. However, gender different impacts of neonatal PCN exposure was observed on the expression of CYP3A11 in a dose dependent manner in mice primary hepatocytes. Notably, attenuated PCN-induced CYP3A11 expression was observed in the primary hepatocytes derived from neonatal PCN exposure mice. Altogether, our study suggested that the persistent alterations of hepatic drug metabolism following neonatal PXR activation are dependent on the drug dose and treatment exposure time at early age. Foundation item: This work was supported by the National Natural Science Foundation of China (Grant U1604163 and 81173127) and China Scholarship Council (201707040007).

2630 CYP1A2 Enzyme Localization in Normal and Diseased Pediatric Livers

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CYP1A2 is a Phase 1 drug metabolizing enzyme whose expression begins between birth and 4 weeks of age, and gradually increases to about half of the adult level by age 10. The enzyme is expressed primarily in liver, where it constitutes 4 - 16% of the total hepatic CYP pool, and is a major determinant of the biotransformation of ~9% of clinically used drugs. Interestingly, CYP1A2 activity is decreased in adults with non-alcoholic fatty liver disease (NAFLD). Considering the increase in the number of medications given to children, as well as the rise in childhood obesity and NAFLD, this study utilized a pediatric liver tissue microarray (TMA) to determine if CYP1A2 abundance and localization was influenced by donor age and disease. The pediatric TMA contained 25 tissues from donors aged 4 months to 18 years old and 5 adult controls. The donor demographics and health data were collected from interviews with next of kin and from hospital records. Three consecutively cut arrays were stained with hematoxylin and eosin (H&E) and Go骂ori’s trichrome for tissue histology, and TRITC collagen, respectively, or with an immunofluorescent anti-CYP1A2 Ab. Images of stained arrays were obtained with a Nikon HCA system and a Hamamatsu Orca Flash 4 camera (CYP1A2) or a Nikon DS-Fi3 camera (H&E and Go吗ori’s trichrome). Disease status was determined from a pathologist’s review of the H&E and Go吗ori’s stains and patient medical records. CYP1A2 protein was detected in all tissues, except in one 4 months old, and increased with age, being greatest in the adult, normal control livers. In pediatric or adult tissues judged to be normal or having minimal pathological findings, CYP1A2 protein was located in zone 3 and 2 hepatocytes. A diffuse pattern of enzyme localization, associated with reduced CYP1A2 level, was seen in tissues affected by necrosis and ischemia. Pediatric NAFLD was also associated with diminished CYP1A2 staining (3 donors, 10 - 14 years old, steatosis 50 - 80%, BMI 32.2 - 32.5). In conclusion, the pediatric liver TMA was a useful tool to elucidate CYP1A2 protein localization in healthy and diseased livers. Moreover, CYP1A2 was reduced in diseased pediatric livers, paralleling what is seen in adults.

2631 Exploration of Hepatocyte Functionality with Tox21 Cell Lines

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The Tox21 Program has investigated thousands of chemicals using cell-based assay systems. These models have been widely utilized for their suspected lack of differentiated tissue functionality (e.g., xenobiotic metabolism). To explore these perceived limitations, Tox21 cell culture models were established and characterized for major human drug metabolizing enzymes (P450, UGT, and SULT). Tox21 cell lines (HeP2, BGI-Luc 4E2, HEK293, Hela, H11CT, ME180, CHO-K1, GH3-TRE-Luc, C3H10T1/2 and MCF-7aroERE) vs. primary human hepatocyte (PHH) suspensions from a 50-donor pool were evaluated drug substrate concentrations to observe detectable metabolite concentrations. These new cell lines were evaluated for glucuronidation and sulfation activity using hydroxocoumarin, biphenol A (BPA) and bisphenol S (BPS). For BPAF and BPS, both glucuronidated and sulfated metabolites were identified in PHHs, with proportionally lower levels of sulfated metabolites for both compounds in HeP2 & HEK293. No metabolites were observed in incubations with MCF-7 cells. Glucuronidated metabolites were not observed in any of the Tox21 cell lines. Tox21 cell models largely failed to support classical hepatic receptor signaling functionality (i.e., AhR, CAR, PXR) compared with sandwich cultures of PHHs growing more than 60% as much as PHHs. Together, these data provides an extensive characterization of the inadequacies of established Tox21 cell lines to model human hepatic xenobiotic metabolism and functional signal transduction pathways. Moreover, the disproportionate metabolite profile for BPS and BPAF towards sulfate conjugates highlights the risks with applying these models with extensively metabolized chemicals towards toxicological prediction.

2632 Glutathione S-Transferase Protection against the Acute Toxicity of Electrophiles is Not Limited to Their Enzymatic Function: Evidence for a Ligandin Contribution


Environmental and endogenous electrophiles cause tissue damage through their high reactivity with endogenous nucleophiles such as DNA, proteins, and lipids. Not surprisingly, organisms have developed numerous protective pathways against these electrophiles, including spontaneous and enzymatic conjugation with glutathione. Enzymatic conjugation is largely mediated by the extensive family of glutathione S-transferase (GST) enzymes, which are present in virtually all living cells and constitute up to 10% of cellular protein content. GSTs perform the first step in the transformation of an electrophile
to its mercapturic acid derivative, a highly soluble metabolite that is rapidly excreted in urine. However, the high expression level of GSTs, their high Kₜ values, and their substrate promiscuity, even between members of the same family, suggest that the protective activity of GSTs may extend beyond their enzymatic function. To address this question we generated a panel of mice lacking various GST gene families. We then compared the impact of these genetic lesions on the ability of the mice to form the mercapturic acid metabo-
lolite of acrylamide and also determined whether these GSTs protect against the tissue damage and metabolic changes that occur secondary to acrylamide exposure. We used ¹H NMR spectroscopy to measure the levels of mercapturic acid metabolites in the urine of mice after an acute acrylamide exposure. With this method, we were able to define the primary GSTs responsible for acry-
amide conjugation; however, the acute toxicity of acrylamide was only ob-
served in mice lacking all three gene families. This finding supports a model in which the cellular protection that is provided by GSTs is not restricted to their ability to conjugate a particular electrophile with glutathione; rather, the often overlooked ligandin function of GSTs can play an essential role in the protection of an organism against toxic electrophiles.

2634 Hepatocyte Hopping of Sorafenib-Glucuronide Is Decreased in Nonalcoholic Steatohepatitis

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Hepatocyte hopping is a hepatocyte-to-blood-to-hepatocyte shuttling route that increases the efficiency of hepatic elimination by allowing for multiple chances at excretion. This process is mainly mediated through sinusoidal efflux of hepatic metabolites by Mrp3 and uptake by Oatp transporters in sequential hepatocytes until eventual biliary efflux by Mrp2. Sorafenib-glucuronide (SFB-G), a metabolite of sorafenib (SFB), is a known substrate of the transporters involved and undergoes hepatocyte hopping to produce relatively efficient biliary elimination. Nonalcoholic steatohepatitis (NASH), the advanced form of fatty liver disease, alters the function of multiple processes that impact metabolism and disposition, including the transporters involved in hepatocyte hopping. Therefore, the purpose of this study was to determine whether NASH significantly affects drug disposition through alterations in hepatocyte hopping. Male FVB and C57BL6 wild-type (WT), Gpat1α/1β cluster knockout (KO), and Mrp2 knockout (mKO) mice were fed a methionine and choline deficient (MCD) diet for 6 weeks to induce NASH. Mice were admin-
istered 10 mg/kg SFB by oral gavage and blood samples were collected via submental bleed at 20 minutes, 1, 2, 4, and 8 hours. Concentrations of SFB and SFB-G in plasma were determined through liquid-chromatography tandem mass spectrometry. Plasma concentrations of SFB-G increased by 108-fold in the oKO-C group when compared to the WT due to the loss of Oatp uptake. In the WT-MCD group, the increased expression of Mrp3 and decreased func-
tion of Mrp2 due to cellular stressors in NASH results in a 165-fold increase in SFB-G concentrations. The additional loss of Oatp transporter uptake along with the alterations to Mrp3 and Mrp2 by NASH in the oKO-MCD group resulted in a 3.2-fold increase in SFB-G plasma concentrations when compared to oKO-C. These results indicate that decreased canalicular efflux by Mrp2 during NASH increases the amount of SFB-G available for hepatocyte hopping. The concurrent increase in Mrp3 efflux and overall decrease in Oatp uptake limits the contribution of downstream hepatocytes to the total elimination of SFB-G. These changes in transporter function significantly reduce biliary elimination and alter drug disposition in NASH by limiting the process of hepatocyte hopping.

2635 Placental BCRP/ABCG2 Transporter Prevents Fetal Exposure to the Estrogenic Mycotoxin Zearalenone

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In the placenta, the BCRP/ABCG2 efflux transporter limits the maternal-to-fetal transfer of drugs and chemicals. Previous research has pointed to the estrogenic mycotoxin zearalenone as a potential substrate for BCRP. Here, we examined the role of the BCRP transporter in the transplacental disposition of zearalenone during pregnancy. In vivo transwell transport assays employing human placenta and BCRP and Mrp7 transfected MDCK cells and human BeWo trophoblasts with reduced BCRP expression were used to characterize the impact of BCRP/BCrp on the disposition of zearalenone. In both in vitro models, the presence of BCRP/BCrp protein increased the basolateral-to-apical transport and reduced the apical-to-basolateral transport of zearalenone over a 2 h period. In vivo pharmacokinetic analyses were then performed on pregnant wild-type and Bcrp⁻/⁻ mice on gestational day 14 after a single tail vein injection of zearalenone. Zearalenone and its metabolite α-zearalenol were detectable in serum, placentas, and fetuses from all animals, and β-zeara-
lenol was detected in serum and fetuses, but not placentas. There were no notable differences in the maternal serum concentration or any of the metabolites between the two genotypes. In Bcrp⁻/⁻ mice, the free fetal concentrations of zearalenone, α-zearalenol, and β-zearalenol were increased by 115%, 84%, and 150%, respectively, when compared to wild-type mice. Concentrations of free zearalenone and α-zearalenol were elevated 145% and 78% in Bcrp⁻/⁻ pla-
centas, respectively, when compared to wild-type placentas. Taken together, these data indicate that the placental BCRP/BCrp transporter functions to re-
duce the fetal accumulation of zearalenone, which may impact susceptibility to developmental toxicities associated with in utero mycoestrogen exposure. This work was supported by the National Institutes of Environmental Health Sciences (Grants ES029275, ES020522, ES050022, ES007148, ES029794), a compo-
dent of the National Institutes of Health.

2636 Developmental Thyrotoxicosis via Dietary Iodine: Identifying Genetic Vulnerabilities Using the Zebrafish Model

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Iodine is an essential precursor to thyroid hormone and is required during development to ensure proper growth. With this in mind, many industrialized countries introduced iodine into the diet through methods such as iodized salt. Because of the high variability in salt intake across the socioeconomic spectrum in children, a modern reevaluation of the consequences of various degrees of iodine overdose, also known as thyrotoxicosis, is being completed. Thyrotoxicosis shares multiple signs and symptoms with attention deficit hyperactivity disorder (ADHD) in children. In addition, variant manifestations of both diseases similarly occur, such as “ADD” and “apathetic thyrotoxicosis.” Exploring definitive differences between these two diseases would provide information in which the cellular protection that is provided by GSTs is not restricted to their ability to conjugate a particular electrophile with glutathione; rather, the often overlooked ligandin function of GSTs can play an essential role in the protection of an organism against toxic electrophiles.

Zearalenone during pregnancy. The examined role of the BCRP transporter in the transplacental disposition of zearalenone during pregnancy. In vivo transwell transport assays employing human placenta and BCRP and Mrp7 transfected MDCK cells and human BeWo trophoblasts with reduced BCRP expression were used to characterize the impact of BCRP/BCrp on the disposition of zearalenone. In both in vitro models, the presence of BCRP/BCrp protein increased the basolateral-to-apical transport and reduced the apical-to-basolateral transport of zearalenone over a 2 h period. In vivo pharmacokinetic analyses were then performed on pregnant wild-type and Bcrp⁻/⁻ mice on gestational day 14 after a single tail vein injection of zearalenone. Zearalenone and its metabolite α-zearalenol were detectable in serum, placentas, and fetuses from all animals, and β-zeara-
lenol was detected in serum and fetuses, but not placentas. There were no notable differences in the maternal serum concentration or any of the metabolites between the two genotypes. In Bcrp⁻/⁻ mice, the free fetal concentrations of zearalenone, α-zearalenol, and β-zearalenol were increased by 115%, 84%, and 150%, respectively, when compared to wild-type mice. Concentrations of free zearalenone and α-zearalenol were elevated 145% and 78% in Bcrp⁻/⁻ pla-
centas, respectively, when compared to wild-type placentas. Taken together, these data indicate that the placental BCRP/BCrp transporter functions to re-
duce the fetal accumulation of zearalenone, which may impact susceptibility to developmental toxicities associated with in utero mycoestrogen exposure. This work was supported by the National Institutes of Environmental Health Sciences (Grants ES029275, ES020522, ES050022, ES007148, ES029794), a compo-
dent of the National Institutes of Health.
Identification of Potential Emerging Chemical Risks in Food from REACH Registered Chemicals

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The European Food Safety Authority (EFSA) is responsible for risk assessment of all aspects of food safety, including the establishment of procedures aimed at the identification of emerging risks to food safety. Data available for REACH registered substances were evaluated by taking the following endpoints into account: (a) environmental release (derived from tonnage and ERC), (b) bio-degradation (BIOWin predictions assessed in a battery approach), (c) bioaccumulation in food (modelled by ACC HUMANeady) and (d) toxicity assessed by classification for mutagenicity, carcinogenicity, reprotoxity and repeated dose toxicity. Two different approaches, weighting scenarios and Pivot table selections, were used to prioritise the potential of toxic chemicals to emerge in the food chain, therefore, to represent a possible risk to food safety. Of the 2336 substances with relevant data available, 212 were identified as priority substances for further evaluation. To confirm the accuracy of this prioritisation, 10 chemicals were selected for in-depth evaluation of information on occurrence, analytical methods and toxicology. The selection focused on chemicals that have not already been assessed in detail by other regulatory bodies and are not included in relevant lists (e.g. the Candidate List of the EU REACH Regulation). For four substances, data on the occurrence in the environment or in food/feed could be found. Enhanced monitoring in food/feed is recommended to better assess their relevance as emerging risks in the food chain. For the remaining six substances, no data on occurrence are available or existing data were considered uncertain. In these cases, monitoring in relevant environmental compartments is recommended to gain more insight into their relevance as environmental contaminants. This study also identified 517 substances for which non-prioritisation solely depended on the fact that they are not classified for the relevant toxicological hazards. If such hazards are identified in the future, these substances would qualify as priority substances within the framework as established in this study.

Occurrence and Co-occurrence of Aflatoxin and Fumonisin in Maize and Maize Products in Nigeria

N. Saha Tura1, L. Liverpool-Tasie1, O. Ademola2, A. Obadina2, and F. Wu4

Aflatoxins and fumonisins are mycotoxins (fungal toxins) that frequently contaminate maize, a key staple food in sub-Saharan Africa. Aflatoxin causes liver cancer and has been associated with acute liver toxicity and immunotoxicity, while fumonisin has been associated with neural tube defects. Both of these mycotoxins have been associated with child growth impairment. Previous animal studies have shown that co-exposure to these mycotoxins may increase hepatocarcinogenicity and other toxicological impacts. This study examines the occurrence and co-occurrence of aflatoxin and fumonisin along the maize value chain in southwest Nigeria. Mycotoxin analyses of maize samples collected from farmers and traders showed that aflatoxin levels increased with increasing time of storage, but fumonisin levels did not increase over time in storage. 90% of the animal feed samples collected from feed mills contained aflatoxin levels exceeding the maximum allowable level set by the US Food and Drug Administration (20 µg/kg). In Nigeria, maize feed vs. non-branded maize snacks, the geometric means of both total aflatoxin and total fumonisin levels tend to be higher in non-branded snacks than in branded snacks. Our study results also indicate that lactic acid fermentation, used to make the traditional maize porridge ogi, significantly reduces levels of aflatoxins and fumonisins. Despite regulatory limits in Nigeria for aflatoxins in maize products, 39.13% of the samples had aflatoxin levels above those limits. Though no regulatory limits currently exist for fumonisins, 9.64% of the samples contained total fumonisin levels higher than the United States regulatory limit. We found that aflatoxins and fumonisins contaminations in maize products extend beyond maize production to stored maize and final food products. Thus, adequately addressing the mycotoxin challenge requires consideration of the entire maize value chain.
**2642 Activated Charcoal as a Broad-Acting Sorbent for Endocrine-Disrupting Chemicals**

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Exposure to hazardous chemicals is of concern during natural and manmade disasters such as floods, droughts, and chemical spills. Chemicals can be distributed throughout the environment following these disasters, contaminate food and water sources, and thus increase the risk of human exposures. These chemicals include a variety of PAHs, industrial solvents, pesticides, heavy metals, and mycotoxins, some of which are considered endocrine disrupting chemicals (EDCs). EDCs are of concern due to their ability to interfere with endocrine systems and contribute to adverse health effects. To minimize exposures to EDCs during disaster events, activated charcoal (AC) can be used as a broad-acting sorbent. Our laboratory tested six medical-grade ACs from various sources (e.g., coconut shell, hardwood, bamboo, and coal) to evaluate sorption potential for EDCs. A model was developed using zearealenone, a food contaminant and known EDC, to evaluate the abilities of various ACs to adsorb onto active surfaces and protect against exposures. Representative chemicals of each class were evaluated, including DDT and glyphosate (pesticides), toluene (solvents), benzo[a]pyrene (PAHs), as well as arsenic and lead (heavy metals). Isothermal analyses using the Langmuir model (q = Qmax * (Kc/C) / (1 + Kc/C)) showed high binding capacities (Qmax) of ACs for these chemicals, with Qmax values ranging from 0.16 to 0.72 mol/kg. A living organism (Hydra vulgaris) was used as an in vivo toxicity indicator and showed that ACs provide approximately 90% protection of hydra from lead toxicity at 0.05% inclusion, and approximately 60% protection from arsenic toxicity at 0.5% inclusion. Further studies evaluated the thermodynamics of sorption reactions and derived therapeutic doses of ACs for use in disaster scenarios. These results suggest that AC can be used as a broad-acting sorbent to facilitate the protection of humans from contaminated food and water during natural and manmade disasters. Supported by NIHES SRP P42 ES0277704.

**2643 Total Arsenic and Speciation Analysis in Seaweeds from South Korea**

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Seaweed, which is rich in dietary fiber and minerals is a popular seafood in South Korea. The annual consumption of seaweed in South Korea is close to 5 kg, and more than 50 kinds of seaweeds are used for food. Toxicity and bioavailability of arsenic containing compounds are closely related to their chemical speciation. Due to the different toxicity between chemical forms, speciation analysis is important for evaluating arsenic uptake. In this study, total arsenic (TAs) and six arsenic species (arsenate (As III), arsenite (As IV), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenobetaine (AsB), and arsenocholine (AsCh)) were analyzed in kelp (n = 33), seaweed (n = 25) seaweed fulvescens (n = 17) and gulfweed (n = 12) samples. South Korea’s Ministry of Food and Drug Safety provided a tAs analysis method and a structural analogue method was used for the pretreatment of the samples. We validated accuracy and resolution using the Langmuir model (q = Qmax * (Kc/C) / (1 + Kc/C)) showed high binding capacities (Qmax) of ACs for these chemicals, with Qmax values ranging from 0.16 to 0.72 mol/kg. A living organism (Hydra vulgaris) was used as an in vivo toxicity indicator and showed that ACs provide approximately 90% protection of hydra from lead toxicity at 0.05% inclusion, and approximately 60% protection from arsenic toxicity at 0.5% inclusion. Further studies evaluated the thermodynamics of sorption reactions and derived therapeutic doses of ACs for use in disaster scenarios. These results suggest that AC can be used as a broad-acting sorbent to facilitate the protection of humans from contaminated food and water during natural and manmade disasters. Supported by NIHES SRP P42 ES0277704.

**2644 How Can Developmental/Reproductive and Endocrine Activity Alerts Be Used in Risk Assessment of Food Contact Chemicals?**

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The uncertainty relating the safety of food contact materials (FCM) triggers increasing public, scientific and regulatory attention. In silico models are an appealing solution for rapid and cost-efficient screening of these chemicals, since for most of them toxicological data is not available. Aside from prediction of chronic toxicity, for their complete hazard assessment, information on their potential developmental and reproductive toxicity (DART) is needed. Existing publicly available predictive in silico tools give a qualitative information related to the presence or absence of DART and/or endocrine effects. This approach is suitable for hazard identification (identification of intrinsic properties of a chemical to cause harm) but it does not give any information about hazard characterization (dose needed to trigger a toxic effect) and even less on its potential health risk. In this context, the present study was aimed at drawing up a fast in silico strategy to predict potential DART and endocrine activity of food contact chemicals in the absence of experimental data. In detail, a stepwise approach, incorporating in parallel structural alerts (SA) and docking on nuclear receptors followed by read-across to quantify the alert, was defined and applied on a diverse data set of 195 food contact chemicals [1] and structural analogues, including 72 chemicals toxicologically characterized for different DART endpoints and/or endocrine activity. The combination of SA and docking gave a full coverage of experimentally positive FCM, with only one misclassification for a FCM structural analogue. Out of 123 characterized chemicals, 12 were prioritized for positive DART and/or endocrine effects. Positive chemicals were promoted for read-across providing a quantitative estimation of the effects. These results showed that in contrast to the use of single models, often addressing specific endpoints and based on very specific and limited applicability domains, our strategy can be applied to different chemical classes and covers a broad range of effects. Most important, it gives a quantitative information to be compared with exposure for the calculation of a margin of exposure in a risk assessment approach. [1] Manganelli, S., et al. (2018). Integrated strategy for mutagenicity prediction applied to food contact chemicals. ALTEX 35, 169–178.

**2645 Monitoring of 8 Polycyclic Aromatic Hydrocarbons in 11 Different Regions of Tap Water in South Korea**

W. Kim1, G. Jeon1, J. Lee2, B. Moon2, and K. Lee1. Korea University, Seoul, Korea, Republic of; and 2 Chung-ang University, Ansan, Korea, Republic of; Sponsor: J. Lee.

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds which are known to relate to damaging the DNA in human tissue and cause cancers or other adverse effects. They are formed as a result of incomplete combustion of organic materials. Once formed, those could contaminate many parts of the environment such as atmosphere, plants, and water. Direct food intake is one of the major routes of exposure to PAHs. During the cooking process, water is the essential ingredient in various recipes. Therefore, it is necessary to monitor PAHs in water. In this investigation, we measured 8 compounds of PAHs (benzo[a]pyrene, chrysene, benzo[b]fluoranthene, benz[a]anthracene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene) in 11 different regional tap water with gas chromatography with mass spectrometry, GC-MS. Benzo[a]pyrene-d12 and chrysene-d12 were used as internal standards. The ultrasonic extraction method was used for the pretreatment of the samples. We validated accuracies and precisions using an intra-inter day test and limit of detection (LOD). As a result, the standards accuracies ranged from 80 to 120 with precision values of 20%. The LOD of all PAHs were ranged between 0.016 to 0.313 ng/L. We could not detect all of 8 PAHs in the tap water in South Korea.

**2646 FDA Assessment of Coronary Heart Disease Risk of Industrially-Produced Trans Fatty Acids in Partially Hydrogenated Oils**


Partially hydrogenated oils (PHOs) are the main dietary source of industrially-produced trans fatty acids (IP-TFA). In 2015, the US FDA determined that there is no longer a consensus among qualified experts that PHOs are generally recognized as safe substances for any use in human foods. In 2018, US
2647 Vomitoxin-Adulterated Adlay Counteracts Toxin-Induced Injuries: New Safety Levels?

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Adlay (Job’s tears, Coix lacerma-job) is a cereal crop that has long been used as traditional herbal medicine and as a highly nourishing food. However, vomitoxin (VT, deoxynivalenol), the most prevalent trichothecene mycotoxin worldwide, frequently spoils grains, including adlay, via fungal infection. Based on the assumption that the actions of VT in the gut could be modified by acetylation and glycoside (modified mycotoxin) of trichothecenes in commodities. We developed a simultaneous analytical method using a multifunctional column for the detection of 7 trichothecenes including four modified forms of 4,15-DAS (4,15-Diacetoxyscirpenol, 7-triacetoxy-3,7-dihydroxy-12,13-epoxytrichothec-9-ene and 4,15-diacetylenalinol), purified from cultures of F. equiseti and T-2 and HT-2 toxins in cereals. The performance of the current method was evaluated, and a total of 248 samples of five different commodities were analyzed for over two years by this method. 4,15-DAS was detected in Job’s tears products, corn flour and azuki beans; however, it was not found in wheat flour or rye flour. The four modified forms of 4,15-DAS were detected in samples of Job’s tears products, contaminated by 4,15-DAS. This is the first report on the quantification of the modified forms of 4,15-DAS in cereals.

2648 A Polyphasic Method for the Identification of Aflatoxigenic Aspergillus from Cashew Nuts

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The invasion of food by toxigenic fungi has become a treat to public health. This study aimed at enumerating the microbial profile, detection of aflatoxin producing genes and quantification of the levels of aflatoxin contamination of cashew nuts meant for human consumption. A polyphasic method of analysis using suitable culture media newly formulated β-Cyclodextrin Neutral Red Desiccated coconut agar (β-CDNRCDA) and Yeast Extract Sucrose agar (YES). Thin Layer Chromatography (TLC), Polymerase Chain Reaction (PCR) and High Performance Liquid Chromatographic (HPLC) method was adopted in determining the aflatoxigenic potential of the isolates, the presence of aflatoxin biosynthetic gene (aflM,aflD, aflR, aflJ and omt-A), and estimation of the total aflatoxin content of the nuts. The fungal counts ranged between 100-1300cfu/g and sixty-three isolates belonging to 18 fungal genera and 34 species were isolated. The Aspergillus spp. were the most frequently isolated (50.79%) while the Trichoderma spp. (15.99%) were the least frequent organisms. Fluorescence production was enhanced on β-CDNRCDA for characterization of aflatoxinogenic fungi. The GFP gene was amplified in all the isolates while aflM, aflR and aflJ gene were each amplified in 77.7% of the isolates and omt-A gene in 70.37%. The aflatoxin content of the nuts were below the 4µg/kg, EU recommended limit for total aflatoxins and ranged from 0.03-0.77 µg/kg. The present work reveals that a single method may not be sufficient for screening for the presence of aflatoxins in foods or isolates, but a combination of different methods will preferably yield a more confirmatory result.

2649 Development of an Analytical Method for the Simultaneous Detection of Modified 4,15-Diacetoxyscirpenol and Its Presence in Japanese Retail Food

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Trichothecene mycotoxins are a group of more than 80 compounds that are mainly produced by the Fusarium species. They are classified into four types (A-D) based on their structure. 4,15-Diacetoxyscirpenol (4,15-DAS) is an A type trichothecene, in which T-2 and HT-2 belong. Regarding the contamination status and analytical methods, there have been many studies on T-2 and HT-2; in contrast, there have been few studies on 4,15-DAS. In 2016 the JECFA evaluated 4,15-DAS and established the total provisional maximum tolerable daily intake (PMDTI) with T-2 and HT-2. However, the contamination of 4,15-DAS in food consumed in Japan has not been surveyed. Recently, the contamination of chemically modified forms of 4,15-DAS with acetylation and glycoside (modified mycotoxin) of trichothecenes in commodities. We developed a simultaneous analytical method using a multifunctional column for the detection of 7 trichothecenes including four modified forms of 4,15-DAS (4,15-Diacetoxyscirpenol, 7-triacetoxy-3,7-dihydroxy-12,13-epoxytrichothec-9-ene and 4,15-diacetylenalinol) purified from cultures of F. equiseti and T-2 and HT-2 toxins in cereals. The performance of the current method was evaluated, and a total of 248 samples of five different commodities were analyzed for over two years by this method. 4,15-DAS was detected in Job’s tears products, corn flour and azuki beans; however, it was not found in wheat flour or rye flour. The four modified forms of 4,15-DAS were detected in samples of Job’s tears products, contaminated by 4,15-DAS. This is the first report on the quantification of the modified forms of 4,15-DAS in cereals.

2650 Toxin Enterosorbs for Inclusion in Food and Water during Emergencies and Natural Disasters

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Following disasters, the mobilization and redistribution of contaminants can increase the risk of exposures to mixtures of hazardous substances and impact the safety of water and food being consumed. In this study, montmorillonite clays have been amended with natural products (i.e., L-carnitine, choline, and chlorophyllin) at 100% cation exchange capacity, or processed to simulate broad-acting carbons. The major goal of this study is to use these sorbents in the diet to reduce human and animal exposures to contaminants at the site of disasters. Based on equilibrium isothermal analysis, thermodynamic studies and mechanistic studies, we have demonstrated significantly increased binding capacities (Qmax, affinities (Kd) and enthalpies (ΔH) of sorption for paragat, glyphosate and benzo(a)pyrene. From dosimetry analyses, we have extrapolated the daily dose of sorbent needed to meet the regulatory levels for individual hazardous chemicals. This information will also facilitate the determination of dosage requirements for enterosorbent therapy. The hydra bioassay was used as a sensitive in vivo indicator of toxicity to confirm the safety and efficacy of sorbents for individual chemicals. Other Superfund chemicals were also shown to be adsorbed with high capacity and affinity, including pentachlorophenol, trichlorophenol, phenol, pyrene, lindane, diazinon, aldicarb and linuron. The hydra method was used to indicate the toxicity of “Designed” mixtures and “Real” mixtures from contaminated water and sediment from Superfund sites, and the ability of sorbents (at low inclusion levels) to protect against these chemical mixtures. We anticipate that a mixture of optimal sorbents developed from this study can be delivered in food,
snacks, condiments, flavored water, sachets or capsules during disasters to reduce human and animal exposure to chemicals and microbes. Supported by NIEHS SRP P42 ES0277704.

**2651 Safety Evaluation of L-Theanine Administered via Hard Chew to Dogs**
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L-theanine has been evaluated in several clinical trials, demonstrating beneficial effects in dogs and cats in recent years. However, specific safety trials of L-theanine in these target species in the public domain are lacking. The present study was designed to evaluate the safety of L-theanine at the recommended dose, and to demonstrate an adequate margin of safety in dogs. Beagle dogs (4 per sex/group; 9.54-14.53 kg) received one or five hard chews orally per day for 28 days; four control dogs were not given any chews. Each 25-g hard chew contained 0.184% L-theanine. Blood was collected on Days 14, 15, and 28. Body weights and clinical examinations were recorded weekly, and animals were returned to stock at study completion. All animals ate the chews spontaneously within one hour, demonstrating good palatability; there was no effect on food consumption. No abnormalities were noted during the clinical observations throughout the study. Mild or moderate symptoms were recorded in some animals, including development of otitis in one animal. Gastroenteritis, constipation, diarrhea, or vomiting were not among the noted abnormal findings. The absence of gastrointestinal symptoms in all groups determined not to be treatment-related and to be within normal physiological range. No treatment-related changes in body weight were observed. While diarrhea was recorded in a majority of the animals, including once in the controls, these effects were also present during the acclimation period, in general, for one dog in each housed pair and were determined not to be dose-related. Some individual hematological values fell outside the physiological ranges. For example, a few individual white-blood-cell counts were low in control-group animals on Day 14 or elevated in high-dose-group dogs before treatment. However, all mean hematological values were within the respective normal physiological ranges. Starting at Day 14, some animals in all groups had globulin values just below the reference range (mean 22 g/L, compared to 25-45 g/L); on Day 28, only the lower dose group remained lower (24 g/L). Similarly, mean chloride values were just over the reference range of 109-122 mmol/L for all groups, and only remained elevated in the 5x dose group on Day 28 (123 mmol/L). Thus, while some minor differences in pre- and post-treatment serum chemistry results were observed, they were determined not to be clinically significant. In conclusion, the L-theanine product was found to not cause adverse effects when administered to dogs orally for 28 days, including up to the targeted margin of safety of 5x the recommended dose.

**2652 Impact of Thermally Abused Oil on Gut Inflammation, Colon Tumorigenesis, and Hepatic Gene Expression in Mice Fed a Standard Diet or the Total Western Diet**
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Deep frying foods damages macromolecule components and contributes to the formation of several toxic compounds known to negatively influence health outcomes, including increased colorectal cancer (CRC) risk. Evidence from in vitro experiments or in vivo animal studies suggest that exposure to oxidized dietary lipids can damage proteins, alter hepatic gene expression, disrupt inflammatory/immunity signaling, or alter glucose metabolism. The objective of this study was to determine the impact of thermally abused oil (TAO) in a healthy rodent diet (AIN93G) or the total Western diet (TWD) for rodents on health endpoints associated with chronic inflammation and CRC. We hypothesized that consumption of TAO would promote gut inflammation and colon tumorigenesis, as compared to diets prepared with fresh oil. The azoxymethane and dextran sodium sulfate model of inflammation-associated CRC was employed with a 2x2 experimental design, where C57BL/6J mice were fed standard AIN93G or TWD, each prepared with either fresh oil or TAO. AIN93G+TAO did not significantly impact any of the measured parameters as compared to AIN93G+fresh oil. Also, dietary exposure to TAO did not enhance colitis symptoms or colon tumorigenesis in mice fed either basal diet. Energy intake in mice provided TWD+TAO was significantly greater than observed in mice fed TAO+fresh oil, nevertheless, this change did not translate to increased body weight or fat mass gain. The observation that TAO appeared to disrupt glucose regulation in the context of a Western-style diet prompted further investigation of hepatic function via gene expression analysis on the Fluidigm Biomark platform. RNA was isolated from hepatocytes using TRI reagent and 48 primers were designed for genes of interest with key regulatory roles. This supplementary analysis will include gene targets associated with insulin homeostasis, lipid metabolism, immune regulation, xenobiotic metabolism, and conjugation, using novel reference genes for normalization.

**2653 Effects of Infant Formula Feeding on Neonatal Mammary Gland Development**
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Soy formulas contain isoflavones, which are structurally similar to estradiol (E2). Given the mammary gland is sensitive to estrogens, there are concerns that soy formula feeding may promote premature mammary development. In this study, we used a neonatal piglet model to identify changes in early mammary gland development in response to different postnatal diets. Groups of female piglets (n=6/group) were fed cow’s milk formula (Milk), soy formula (Soy), or breastfed with the sow (Sow) postnatal day 2-21. To assess directly the effects of estrogen or isoflavone on mammary development, additional groups were fed Milk supplemented with estradiol (Milk+E2) or with genistein (Milk+G). In the Milk+E2 group, serum E2 concentration was significantly higher (10- to 20-fold) relative to other diet groups. Terminal end bud numbers were significantly increased in the Milk, Soy and Milk+G groups relative to Sow or Milk+E2 groups. Microarray analysis identified 130, 253, 125, and 170 differentially-expressed transcripts (±2-fold, P<0.05) between Milk, Milk+E2, Milk+G and Soy groups, respectively, relative to the Sow group. Functional annotation analysis indicated formula feeding alters gene expression in pathways associated with cell proliferation and apoptosis. Additional pathways that were identified, cell morphogenesis and differentiation and hormone signaling were only associated with the Milk+E2 group. We also confirmed several down-regulated microRNAs including miR-128, miR-1 and miR-422b (2.4- to 10.2-fold) in the formula groups compared to the Sow control; these have been implicated in regulation of cellular apoptosis and proliferation. The changes in mRNA expression of Wnt2b, cyclin D and n-myc indicated a heightened proliferative state in mammary glands of all formula groups compared to the Sow group. E2-responsive genes, Pgr, Tgfβ2, Rerg, were significantly upregulated in the Milk+E2 group relative to all other groups. Our data suggest that dietary exposure to soy isoflavones during infant feeding does not elicit an estrogenic response in the mammary gland. Still, formula feeding may initiate proliferative pathways independent of estrogenic signaling.

**2654 Application of Sphingolipids as Biomarkers of Fumonisins Exposure in Kenyan Children**
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Fumonisins (FNs), a group of mycotoxins produced mainly by several Fusarium species, are ubiquitous food contaminants, especially for corn and corn products. Fumonisin B1 (FB1) caused severe toxicities in farming animals, induced kidney and liver tumors in rodents and fish, and has been associated with many human adverse health effects, including esophageal cancer. IARC has categorized FB1 as possible human carcinogen (Group 2B). Inhibition of ceramide synthesis and disruption of sphingolipids metabolism have been well studied as the major mechanisms of FB1-induced toxicity. Dynamic changes of sphingolipids, specifically increases in sphinganine (Sa) and decrease in sphingosine (So) levels as well as their ratio have been proposed to be the major indicators of FB1 exposure in human subjects. The primary objective of this study was to determine the feasibility of using sphingolipid analysis as a biomarker for Fumonisin exposure in children. A total of 284 urine samples were collected from children age 1 to 14 years in Kenya, a high mycotoxin exposure region. Exfoliated cells from urine were isolated, processed and sphingolipids quantified by High Pressure Liquid Chromatography with fluorescence detection. Sa and So were detectable in 95.07% and 98.94% of the urine samples respectively. All non-detectable samples were from male subjects. The levels of Sa, So and Sa/So ratio and assessed FN exposure in Kenya children who consume maize as a staple diet. A total of 284 urine samples were collected from children age 1 to 14 years in Kenya, a high mycotoxin exposure region. Exfoliated cells from urine were isolated, processed and sphingolipids quantified by High Pressure Liquid Chromatography with fluorescence detection. Sa and So were detectable in 95.07% and 98.94% of the urine samples respectively. All non-detectable samples were from male subjects. The levels of Sa, So and Sa/So ratio and assessed FN exposure in children. 389

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2655  Aflatoxin B1-Induced Alterations on Gut Microbiota-Dependent Nutritional Metabolome in F344 Rats

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Human gut-microbiota serves as the key hub of initial nutritional provision. Our previous study has demonstrated that aflatoxin B1 (AFB1), a major contributor to the AFB1-associated toxic side effects, can be well separated by LemonGlyerol treatment using weighted Unifrac analysis. Furthermore, GMB analysis of the feces at week 4 indicated that the GMB metabolic patterns, with the most prominent difference observed in the treatment groups, were aligned, and 60 of the top 100 differential features were imputatively identified from NIST database. Bioinformatic analysis demonstrated that the major metabolic pathways disrupted by AFB1 include: valine-leucine metabolism, bile acid and steroid synthesis, GTP Synthesis, N-acetyl-D-glucosamine synthesis, and carbohydrate conversion. For data collected with HRLC-LTQ Orbitrap MS, 3925 MS1 features were aligned using MZmine, whereas 2498 MS2 features (234 groups) were aligned using XCMS. A total of 86 metabolites were identified from these features by searching Human Metabolome Database (HMDB), MassBank, and METLIN databases. The major metabolic pathways affected by AFB1 include: retinol metabolism, glycerolipid metabolism, penelope and glucuronate interconversion, glycerophospholipid metabolism, arginine and proline metabolism. The categories of the altered metabolites/nutrients include carbohydrates, amino acids, bile constituents, phospholipids, glycerolipids, and fatty acids. Interestingly, a number of carbohydrates were accumulated in the feces, such as glucose (2.72 fold), rhamnose (3 fold), fructose (3.6 fold), turanose (2.03 fold), galactose (2 fold), galactitol (1.53 fold), glycerol (1.35 fold), ribose (1.26 fold), 3-beta-D-galactosyl-sn-glycerol (1.36 fold), arabinose (1.39 fold), and trehalose (12.23 fold). These abnormal alterations suggest the impairment of carbohydrate associated metabolisms. Taken together, these results indicated that the impairment of gut-microbiota dependent nutritional metabolome may be a major contributor to the AFB1-induced adverse health outcomes in animals and humans.

2656  Modulation of Microbiome, Glucose Homeostasis, Behavior and Cognition in Non-obese Diabetic Mice Following Daily Dosage of Lemonglycerol Dietary Supplement

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The Lemonglycerol (HGG Research LLC) has been claimed to support gut and brain health by relieving vertigo, dizziness, headaches, dry throats, and nausea. However, no preclinical studies have been conducted to confirm the success of this dietary supplement. We have hypothesized that brain function can be beneficially regulated by Lemonglycerol in order to maintain the gut-brain axis, which states that the gut and the brain can communicate and affect each other in a number of ways, including gut microbiota (GBM) modulation. To test this hypothesis, non-obese diabetic (NOD) mice had been dosed daily with vehicle (water), lemon extract, food grade glycerol solution and LemonGlycerol (0.05 mL per day) for a total of 6 months. In addition to monitoring the body weight and glucose levels, the tail suspension and Y-maze tests, which assess the depression and cognitive behaviors, were conducted. Our findings suggested that there were no significant effects on glucose homeostasis of the mice and the cognitive behavior at week 17. Despite not reaching the level of statistical significance, there was a decreasing trend of the immobilization time in the tail suspension test for the treatment groups, with the most prominent difference observed in the Lemonglycerol group. This suggested a potential decrease in depression. Furthermore, GMB analysis of the feces at week 4 indicated that the GBM could be well separated by LemonGlycerol treatment using weighted Unifrac with increases of Bacteroides, Desulfovibrioionales and Y52, and decreases of Lactobacillales and Streptophyta at the order level. This anti-inflammatory response was further supported by increases in the abundance of the genera Prevotella, Ruminococcus and Staphylococcus. Further analysis of the pathways and functional metagenomics using Tax4Fun may be able to shed more light on this new prebiotic that might help an array of people suffering from various symptoms listed above and reduce the intake of medications with toxic side effects. Supported in part by NIH R41AT009523.

2657  Safety Paradigm for Evaluation of Food Additives/Ingredients Produced Using Genetically-Modified Microorganisms

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The safety assessment of genetically-modified (GM) foods, which contain viable or non-viable GM microorganisms (GMMs), have been discussed extensively among scientific and regulatory bodies; however, these evaluations have explicitly excluded consideration of high purity additives/ingredients produced using GM technology. To date, authoritative safety paradigms for evaluation of these ingredients have not been published. A guiding principle in the evaluation of GM foods produced using GM technology has been the concept of ‘substantial equivalence’, which embodies the idea that, if the food derived from a GMM is demonstrated to be substantially equivalent in composition and nutritional characteristics to its conventional counterpart, the history of safe consumption of the conventional counterpart can be extended to the GM ingredient. While substantial equivalence can serve as a baseline for the risk assessment of a food additive/ingredient produced using GM technology, additional considerations are necessary in the safety evaluation of these ingredients. Accordingly, a safety paradigm has been proposed to evaluate the safety of food ingredients produced using GMMs. The first step in this paradigm involves evaluating the safety of the food ingredient using the same scientific procedures approach that is applied to non-GM ingredients, and where applicable, involves a ‘substantial equivalence’ approach to evaluate ingredients with a conventional counterpart that have a history of use or have been previously evaluated in animal studies. The second step assesses the potential risks of the host organism lineage and the effects of the introduced genetic transformations, e.g., have genetic modifications introduced pathogenic, toxicogenic, or other undesirable phenotypes, such as antibiotic resistance to the strain? The third step focuses on the safety of the recombinant protein expression products produced by the GMM, and includes bioinformatic evaluation of recombinant proteins for homology, immunoreactivity, and allergenicity. Overall, this strategy is particularly applicable to situations where traditional safety studies may not be feasible (e.g., vitamins), and promotes ethical safety evaluation practices to avoid unnecessary and redundant animal testing of GM additives/ingredients that have an equivalent comparator in the food supply.

2658  Exposure Assessment of Glyphosate in Breakfast Cereals in the US


Glyphosate is one of the most widely used herbicides for agriculture and forestry applications, as well as on lawns and gardens in the United States. This broad-spectrum non-selective herbicide inhibits 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, a key enzyme necessary for protein synthesis in plants. In March 2015, the International Agency for Research on Cancer (IARC) classified glyphosate as a Group 2A "probably carcinogenic to humans" carcinogen. Subsequently, it was listed under California’s Proposition 65 as a chemical “known to the state to cause cancer” in July 2017. However, the US Environmental Protection Agency (US EPA) reported that glyphosate was “not likely to be carcinogenic to humans,” and the Food and Agriculture Organization (FAO) as well as the World Health Organization (WHO) reported that “glyphosate is unlikely to be genotoxic at anticipated dietary exposures.” There have been recent reports indicating concern regarding glyphosate in cereal. The purpose of this study was to assess the potential cancer risks associated with consumption of glyphosate in different grain-based cereals by age groups by comparing to the Office of Environmental Health Hazard Assessment’s (OEHHA) proposed No Significant Risk Level (NSRL) of 1100 µg/day for glyphosate. Glyphosate intake was estimated using the mean and maximum estimates of consumption, reported by the Environmental Working Group (EWG). Glyphosate exposure from consumption of all cereal types (conventional and organic) for all age groups (6 months to >70 years) ranged from 0.0575 to 8.14 µg/day and 0.0575 to 133 µg/day for mean and maximum estimates of consumption, respectively. Overall, mean and maximum glyphosate exposures through cereal consumption were lower in organic cereals (mean = 0.292 - 8.14 µg/day; max = 0.0575 - 2.62 µg/day) than conventional cereals (mean = 0.0575 - 0.161 µg/day; max = 0.0575 - 0.161 µg/day) for all age groups (6 months to >70 years) ranged from 0.0575 to 8.14 µg/day and 0.0575 to 133 µg/day for mean and maximum estimates of consumption, respectively. Overall, mean and maximum glyphosate exposures through cereal consumption were lower in organic cereals (mean = 0.292 - 8.14 µg/day; max = 0.0575 - 2.62 µg/day) than conventional cereals (mean = 0.0575 - 0.161 µg/day; max = 0.0575 - 2.62 µg/day) for all age groups. Daily glyphosate doses associated with mean and maximum cereal consumption rates of all categories of cereals are well below OEHHA’s NSRL. The cancer risk from glyphosate exposure from cereal is not likely to be of concern in the United States. Future studies should focus on characterizing total glyphosate exposure from multiple food sources such as wine, fruit juices, legumes, and honey.
2659 The Effects of Activated Carbon on Vitamin K and Blood Coagulation in Rats
Activated carbon is widely used to treat intoxications caused by toxic chemicals taken orally, toxins generated in the gastrointestinal tract, drug overdose etc. However, activated carbon can adsorb not only toxins but also nutrients including enzymes, vitamins and minerals, and the overdose has the potential for nutrient deficiency. Among such nutrient compositions, vitamin K plays an important role in the maintenance of blood coagulation, and the deficiency of vitamin K can cause hemorrhage. In this study, we examined the effects of activated carbon on vitamin K and blood coagulation in rats. Activated carbon was administered orally (dietary administration) to male and female rats at 0 (control), 1.0 and 5.0 w/w% for 2 weeks (6 animals/group); the high dose provides more than 10- to 100-fold margin of the clinical dose (2 to 20 g/day). Clinical signs, body weight, food consumptions, hematology (prothrombin time (PT) and activated partial thromboplastin time (APTT)), blood chemistry (plasma vitamin K concentration), and necropsy were performed. There were no abnormalities in clinical signs, body weights and necropsy. An increase in food consumption, prolongation of PT and APTT, and a decrease in plasma vitamin K concentration were noted in both sexes of the treated groups with dose-dependent manner. The degree of the change in PT and APTT was larger in male rats than in female rats; it is known that male rats are more susceptible to vitamin K deficiency than female rats. In addition, we confirmed that activated carbon has adsorption capacity for vitamin K in vitro. In conclusion, activated carbon has adsorption capacity for vitamin K and the large excess interference with absorption of vitamin K from diet potentially induces blood coagulation disorder.

2660 Low-Level of Total and Arsenic Speciation of Cereal Grains in South Korea
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Sponsor: J. Lee
Arsenic (As) compounds have different toxic effects on human depending on chemical species with unique characteristics. Inorganic arsenic (iAs, iAs = arsenite (As (III)) and arsenate (As (V))) is more toxic than organic arsenic and is known to affect cardiovascular disease and cancer. The most important contributor to dietary arsenic to As is cereals. Cereals are consumed as important foods that can provide calories and protein to human. Nevertheless, there is a lack of research on the concentration of arsenic species in various cereals. In this study, total arsenic (tAs) and six arsenic species (As (III) and As (V), monomethylarsonic acid (MMA), dimethylarsonic acid (DMA), arsenobetaine (AsB), and arsenocholine (AeC)) were analyzed in adlay (n = 5), oats (n = 13), glutinous millet (n = 15) and sorghum (n = 15) samples. South Korea’s Ministry of Food and Drug Safety, MFDS provided a tAs analysis method and a method for separating 6 species of arsenic. For the present study, tAs was analyzed using inductively coupled plasma-mass spectrometry, ICP-MS, and 6 species of arsenic were analyzed by high-performance liquid chromatography coupled with inductively coupled plasma-mass spectrometry, HPLC-ICP-MS. In our study, tAs and iAs concentration were at a low level. It is also interesting to note that a positive correlation was observed between tAs and iAs concentration in cereals. Also, iAs fractions were dominant in all cereal samples. The main arsenic species found in cereals were As (III), followed by As (V), DMA and MMA, AsB and AeC were not detected. The result of this study suggests a valid method for determining exposure and potential risk of arsenic species.

2661 Evaluation of Exposure to Mycotoxins through Fruit Juices Consumption by Chromatographic Methods Coupled to Mass Spectrometry in Tandem
The Rapid Alert System for Food and Feed (RASFF) of the European Union places mycotoxins in the second position according to the total number of hazard notifications. Fruits are susceptible to attack by pathogenic fungi, due to their high sugar content, ideal water activity and nutrient composition. Mycotoxin occurrence in fruits is led by various genera of fungi such as Alternaria, Fusarium, Penicillium, Aspergillus. The objective of this study was to evaluate the presence of 30 mycotoxins (AFB1, AFB2, AFG1, AFG2, AOH, AME, OTA, FB1, FB2, ENNA, ENNA1, ENNB, ENUB, BEA, STG, ZON, aZAL, βZAL, αZOL, βZOL, DON, 3-ADON, 15ADON, DAS, NIV, FUS-X, NEO, PAT-T2 and HT-2 toxins) in fruit juice samples (n=80) by Dispersive Liquid-Liquid Microextraction (DLLME) and determination by gas and liquid chromatography-tandem mass spectrometry. The methods accuracy was evaluated by recovery assays at three concentration levels, and precision, expressed as the intra- and inter-day relative standard deviations (RSD%). Good validation results in terms of recoveries (63-113%), reproducibility (RSDs <15%) and repeatability (RSDs>20%) were reached. Moreover, limits of detection (0.03-2.34µg/L) and quantitation (0.1-7.85µg/L) achieved were lower than the legal limits. Matrix effect was evaluated and matrix-matched calibrations were used for quantitation. Results showed presence of at least one or two mycotoxins in 69% and 8% of analyzed samples. The mean contamination level of AFB1, AFB2, AFG2, OTA, βZAL, PAT, AME and AOH were 9.36, 4.49, 3.75, 5.43, 23.22, 28.63, 8.54 and 207.01 µg/L respectively. The estimated daily intake (EDI) of the child and adult population was assessed using the deterministic approach, through evaluation of mycotoxin data contamination of fruit juices and consumption. EDIs resulted below the TDIs for the selected mycotoxins. The obtained results indicated that mycotoxins are presents in different fruit juice samples and highlighted the need to perform continuous efforts to ensure food safety. Acknowledgements. Spanish Ministry of Economy and Competitiveness (AGL2016-77610-R) and Government Scholarship program “Carlos Antonio López - Paraguay.”

2662 Toxic Tall Fescue Grazing during Fluctuating Environmental Conditions: Impact on the Metabolome, Microbiome and Their Interaction
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Fescue toxicosis (FT) is a livestock disease resulting from grazing tall fescue infected with the ergot alkaloid-producing Epiclhoeae coenophialum and it has a billion dollar negative impact to the U.S. beef industry. The adverse impact of heat stress (HS) on livestock production is an increasing concern and it costs an additional $369 million to US beef producers. Although previous studies showed HS (i.e., high temperature-humidity index (THI)) exacerbates FT signs, the effects of FT and changing THI conditions on metabolic homeostasis, microbiota contribution to metabolic perturbations, and metabolome-microbiome interactions, are unknown. Thus, we utilized fecal 16S rRNA sequencing and plasma and urine high-resolution metabolomics to investigate the effects of toxic tall fescue (E+) grazing and fluctuating THI in Angus steers over a 26-day grazing trial. Grazing E+ fescue decreased weight gain and induced significant changes in amino acid, biotinoprotein, and energy-producing metabolic pathways in both plasma and urine across the trial, with significant interactions between glutamate, tyrosine, and tryptophan metabolites affected by E+ grazing. E+ grazing during elevated THI resulted in significantly decreased biotinoprotein and glutathione (plasma) and increased carbohydrate and butyrate (urine) metabolism. E+ grazing had a main effect on the fecal microbiota, namely on the Ruminococcaceae, Lachnospiraceae, and Coriobacteriaceae families, with these families having OTUs significantly correlated with THI (r > 0.4; P < 0.05). Differential network analysis correlated microbial OTUs were significantly associated (r > 0.7; P < 0.05) with metabolites involved in androgen/estrogen and fatty acid metabolism in E+ steers. As revealed by predictive metagenomics, metabolomes involved in bile acid biosynthesis and amino acid metabolism were decreased by E+ grazing. Overall, these data indicate that effects of E+ grazing on the metabolome and microbiome are altered by THI, which may explain differences in signs or severity of FT throughout the calendar year. Support: USDA (NIFA), Grant Number 67030-25004 to NMF.

2663 In Vivo Genotoxicity Study of Cylindropermopsis by the Comet Assay
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Cylindropermopsis (CYN) is a biotoxin produced by different cyanobacteria species which has hepatotoxic, cytokotoxic and neurotoxic effects in aquatic organisms and mammals. Its ability to bioaccumulate in tissues of different organisms intended for human consumption may have implications in food safety. In this sense, according to the recommendation from the European Food Safety Authority (EFSA) for the genotoxic assessment of substances in food, the in vivo approach is needed to confirm the previous contradictory in vitro results obtained. In the present work, the in vivo genotoxicity of CYN in Wistar rats was evaluated in liver, stomach and blood by the comet assay. Male animals were exposed orally by gavage to CYN (7.5, 23.7, and 75 µg/kg body weight), and the assay was performed according to the recommendations of OECD guideline 489. This assay provides information about mecha-
nisms of genotoxicity since it can detect DNA breaks as well as oxidative DNA damage when the modified enzyme-comet assay is used (Endonuclease III (Endo III) and Formamido pyrimidine glycosylase (FPG)). The results for the standard comet assay revealed no significant increases in DNA strand breaks at any dose assayed. Similarly, post-treatment with Endo III or FPG showed no differences between treated and untreated groups. Thus, CYN does not induce genotoxicity in vivo with the comet assay. However, further research is required to cover all genetic endpoints required by EFSA. Acknowledgment: Spanish Ministerio de Economía y Competitividad for the project AGL2015-64558-R, MINECO/FEDER, UE, and for the grant DPI (BES-2016-078773) awarded to Leticia Díez-Quijada Jiménez.

Magnesium Biotinate (MB) is a novel, highly soluble salt of biotin. The genetic toxicity profile of MB was assessed by performing the Ames assay and in vitro micronucleus assay. Additionally, systemic toxicity of MB was investigated using single and repeat dose toxicity studies. No evidence of mutagenicity was observed in the Ames assay. The in vitro micronucleus test showed no evidence of clastogenicity. Acute oral toxicity testing established a LD50 greater than 5000 mg/kg of body weight. No effects on body weight parameters were observed after 14-days of oral administration at doses up to 2500 mg/kg/day; therefore, a 90-day study was conducted at dose levels of 120, 600 and 2500 mg/kg/day. In the 90-day study, one female at 2500 mg/kg/day was euthanized for humane reasons on Study Day 63 and exhibited hunched posture, facial/abdominal/ano-genital staining, diarrhea, red nasal discharge, reduced food consumption, reduced fecal volume, and thin appearance. This animal had macroscopic and microscopic evidence of gastric ulceration and lymphoid and bone marrow depletion. For the surviving animals, there were no clinical or ophthalmic signs. A slight decrease in body weight and body weight gain was observed for males at 2500 mg/kg/day; however, due to the magnitude and lack of correlation with clinical or histopathological findings, the decrease was interpreted not to be adverse. There were no test substance-related changes in coagulation and urinalysis parameters. Slight increases in sodium, potassium and/or chloride were observed in males at all dose levels and in females at greater than or equal to 600 mg/kg/day, but were not associated with changes in other serum chemistry indices by in vitro endpoints of toxicity. Given the hydrophobic nature of most of these compounds, the dose of 100 µM was the highest achievable dose to accommodate the no-observed-adverse-effect-level for MB, administered orally for over 90 days, which was determined to be 600 mg/kg/day.

Chloropropanols are chemical contaminants that can be formed during industriual processing of foods, such as lipids used in commercially available infant formula in the United States. Studies have shown that the most common chloropropanol, 3-monochloropropene-1,2-diol (3-MCPD), and lipid ester derivatives of 3-MCPD may have the capacity to be carcinogenic and induce injury to the kidneys and reproductive organs in animal models. To investigate the safety of the most abundant chloropropanols in infant formula, we asked whether the proximal tubule cells of the kidney would be vulnerable to their effects in a direct exposure model in vitro. Using the established human kidney proximal tubule cell line, HK-2, we performed 24-hour treatments with 3-MCPD and select mono- and di-esters derived from palmitate, oleate, and linoleate. Direct exposure to HK-2 cells at treatment doses ranging from 0 to 100 µM was performed to evaluate effects on cell viability, mitochondrial health, reactive oxygen species (ROS) production, and other endpoints of toxicity. Given the hydrophobic nature of most of these compounds, the dose of 100 µM was the highest achievable dose to accommodate our in vitro cell culture model. For each test compound, cell viability only became compromised at high treatment doses (50-100 µM range; P<0.05). These high dose effects were verified by fluorescence imaging of cells treated and stained with Hoechst 33342 and Propidium Iodide. Our study showed that the mode(s) of cell death may not have directly involved disruption of mitochondrial membrane potential, since mitochondrial integrity was not strongly affected except at high doses of the mono-ester of linoleic acid (1-Li). Moreover, significant production of ROS molecules was only detectable at low doses of the 3-MCPD di-esters of the oleic and linoleic acid combinations (O-Li and L-Li; P<0.05). Since chloropropanols reportedly inhibit cell metabolism through interference with glycolysis, we also tested the extent of this
2672 Influence of Serum Lutein and Zeaxanthin on Determination of L-Lysine by Raman Spectroscopy

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The Food and Drug Administration does not regulate dietary supplements meaning the supplement companies are responsible for ensuring there are no dangerous contaminants and have the suggested dosage of active ingredients. This study was to compare the amount of L-Lysine in nine dietary supplements to the amounts on the packages using Raman Spectroscopy. Raman Spectroscopy is a scattering method that measures the vibrational modes of molecules. This method was used as opposed to the other analysis methods due to relative speed of detection. Dietary supplements were chosen based on their availability in nearby stores and by Amazon’s Most Recommended List. For the standards, 1.5 g, 2.0 g, 4.0 g, 6.0 g, and 8.0 g of L-Lysine Hydrochloride were measured into 25.0 mL volumetric flasks. Based on the amounts of L-Lysine claimed on the packings, which were either 500 mg or 1000 mg per pill, the number of pills to deliver approximately 5.0 grams of the L-Lysine were crushed and dissolved. Standards and samples were prepared by either dissolving in distilled water or in 1.0 X 10^{-3} M HCl. HCl was used as a closer approximation to stomach conditions. The supplements were then filtered and scanned by PHARM-ID Raman spectrophotometer. The results showed that supplements dissolved in the water have lower amounts of lysine detected than the supplements dissolved in 1.0 X 10^{-3} M of HCl. Overall, the amounts of L-Lysine in the supplements were 26% higher for water and 34% higher than the stated value for HCl.

2668 Virulence Evaluation of Salmonella enterica Isolates Containing Incompatibility Group I1 (IncI1) Plasmids from Food Animal and Human Sources

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Salmonella enterica is a leading foodborne pathogen in the U.S. Mobile genetic elements, such as plasmids, can potentially increase their ability to infect and persist in hosts. IncI1 plasmids are widely distributed in Salmonella from food animal sources. These types of plasmids often encode antimicrobial resistance; however, little is known about their impact on the virulence. To assess the potential impact of the plasmids on virulence, 46 IncI1-positive Salmonella isolates from food animal and human sources were evaluated for their abilities to invade and persist for 48 hours in Caco-2 human intestinal epithelial cells. For the virulence studies, Caco-2 cells were grown to confluence, infected with Salmonella isolates and incubated for both one and 48 hours for the invasion and persistence assays, respectively. Additionally, Salmonella isolates were assessed for their ability to produce colicin toxins and inhibit other bacteria. All isolates infected Caco-2 cells after one hour incubation and persisted in the cells at 48 hrs. Persistent cell counts were observed to be significantly higher than invasion assay cell counts in 26% of the isolates. Overall, by (n=36) of Salmonella isolates were able to inhibit growth of at least one E. coli strain. In this study, isolates carrying IncI1 plasmids were able to invade and persist in elevated levels in the intestinal epithelial cell model and able to produce colicin toxins that may provide a selective colonization advantage. This study provides the foundation for additional studies to refine the contribution of the specific IncI1 plasmid-associated genes to virulence. Identification of genetic determinants relevant for increased virulence and toxin production would help to design screening tools for foodborne pathogens and enhance food safety.

2669 Evaluating the Significance of Immunomodulatory Effects Reported for Food Ingredients Undergoing Generally Recognized as Safe (GRAS) Evaluations

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The functioning of the immune system is influenced by several factors including the diet. When food ingredients and functional foods are associated with immune modulation, there is not always a clear understanding as to whether these changes are indicative of an adverse, beneficial, or neutral effect. From a regulatory perspective, the US FDA has received some Generally Recognized as Safe (GRAS) notifications reporting immunomodulation, to which the Agency appears to have responded with questions regarding the safety of these effects. The purpose of the current work was to identify the immunomodulatory changes reported in these notifications and to distinguish which effects should be considered innocuous, versus those that could be indicative of safety concerns. Specific immunomodulatory effects were identified from publicly available and confidential GRAS evaluations. Relevant toxicology data were systematically identified for each immunomodulatory factor to determine their significance in terms of safety in food. Increased numbers of blood dendritic cells, CD8+ cytotoxic T cells, and natural killer cells were identified as common immunomodulatory effects reported in GRAS evaluations of certain probiotic and fungal derived products that were flagged as potential safety concerns. These findings, however, were not accompanied by significant changes in any other safety-related endpoints. Slight increases in cells with inflammatory potential are unlikely to be of immediate physiological significance without supporting evidence such as elevated levels of inflammatory cytokines (e.g., TNFα) or indication of tissue injury (e.g., increased ALT levels). However, it appears that the safety issue relates to the potential long-term consequences of sustained immunostimulation, which is difficult to completely refute with short-term toxicology data. Although GRAS evaluations of certain probiotic and fungal derived products demonstrate a slight increase in the number of cells with inflammatory potential, these effects are unlikely to be of immediate physiological significance. Further investigations on the consequences of sustained long-term low-level stimulation of the immune system from food is warranted to overcome safety concerns.

2670 Determination of L-Lysine by Raman Spectroscopy

E. Holland, and R. Bright, Fort Valley State University, Fort Valley, GA.

The Food and Drug Administration does not regulate dietary supplements meaning the supplement companies are responsible for ensuring there are no dangerous contaminants and have the suggested dosage of active ingredients. This study was to compare the amount of L-Lysine in nine dietary supplements to the amounts on the packages using Raman Spectroscopy. Raman Spectroscopy is a scattering method that measures the vibrational modes of molecules. This method was used as opposed to the other analysis methods due to relative speed of detection. Dietary supplements were chosen based on their availability in nearby stores and by Amazon’s Most Recommended List. For the standards, 1.5 g, 2.0 g, 4.0 g, 6.0 g, and 8.0 g of L-Lysine Hydrochloride were measured into 25.0 mL volumetric flasks. Based on the amounts of L-Lysine claimed on the packings, which were either 500 mg or 1000 mg per pill, the number of pills to deliver approximately 5.0 grams of the L-Lysine were crushed and dissolved. Standards and samples were prepared by either dissolving in distilled water or in 1.0 X 10^{-3} M HCl. HCl was used as a closer approximation to stomach conditions. The supplements were then filtered and scanned by PHARM-ID Raman spectrophotometer. The results showed that supplements dissolved in the water have lower amounts of lysine detected than the supplements dissolved in 1.0 X 10^{-3} M of HCl. Overall, the amounts of L-Lysine in the supplements were 26% higher for water and 34% higher than the stated value for HCl.

2671 Dried Pig Faeces: Impacts on Nutrient Utilization, Histology and Haematology of Clarias gariepinus

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Improper utilization and disposal of pig faeces in pig farms have been a great concern due to their hazards and risks they constitute to the environment. This situation calls for conversion of animal waste to wealth through effective utilization of pig faeces for fish feed formulation. This project was therefore designed to provide information on safe level of Dried Poultry Faeces (DPF) on growth, histology and haematology of Clarias gariepinus. A total number of one hundred and fifty samples of C. gariepinus were collected for the feeding trial experiment. Growth, histology, and haematology of the fish samples were determined according to standard methods. Data obtained were subjected to statistical analysis using ANOVA and Duncan multiple range test was used to separate the means. The Mean Weight Gain revealed a significant increase in T3 (69.40g) among the treatments, while 100% inclusion of DPF exhibited lowest value (21.07%). The Packed Cell Volume, Haemoglobin and Red Blood Cell values obtained decreased with increase in DPF inclusions, while an increase in these parameters were observed in T4 (26.50%). White Blood Cell count was significant in T3 (16,150±2.07g/l) and decreased in T4(15,800±2.10g/l) and T2(12,650±0.85g/l) respectively. Histological results confirmed the absence of lesions in the heart, gills and the intestine of fish samples. Lesions was severed in the liver in T4 and T5. The findings of this study revealed that 50% inclusion of DPF is the safe level for growth and survival of C. gariepinus. Since the histological results also revealed no lesions in all the vital organs, except the liver that had severe lesions, DPF can be recommended as an alternative feed ingredient that could substitute fish meal in the fish diet.

2672 Influence of Serum Lutein and Zeaxanthin on Select Cancer Mortality: An 18-Year Follow-Up Cohort Study

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Lutein and zeaxanthin (L&Z) are members of the carotenoid family. Food sources of L&Z include a variety of fruits and vegetables, with green leafy vegetables being the richest source. Observational studies indicate L&Z reduce risk for chronic diseases including cancer. L&Z possess important antioxidant properties, promote anti-carcinogenic effects, and have immune modulatory effects and apoptosis-inducing capabilities. To our knowledge, influence of
L&Z on cancer mortality has not yet been characterized. Thus, our objective was to evaluate potential association between L&Z and all-cancer (i.e., breast, colorectal, lung, and prostate cancers) mortality. This retrospective cohort study was conducted with 14,358 adults who participated in Phase II of the National Health and Nutrition Examination Survey III (NHANES III). The dataset served as baseline and was correlated with the National Death Index database for an 18-year (1998–2006) follow-up study. Hazard ratios (HRs) for cancer-related deaths for individuals with high, medium, and low serum L&Z levels were calculated using the Cox Proportional Hazards Regression Model. Unadjusted HRs of all-cancer deaths associated with low serum levels (25% cutoff) of L&Z were 1.32 (95% CI=1.08-1.62) and 1.00 (ref). Adjusted for multiple risk factors (age, sex, race/ethnicity, education, alcohol intake, and cigarette smoke), HRs for all-cancer deaths were 1.18 (95% CI=0.95-1.45) for low serum levels of L&Z and 1.00 (ref). Adjusted HRs for all-cancer deaths, using a 3-level categorization (not adjusting for fruits and vegetables), were 1.50 (95% CI=1.19-1.90) for low vs. high and 1.40 (95% CI=1.18-1.65) for medium vs. high; colorectal cancer mortality was 2.49 (95% CI=1.44-4.22) for medium vs. high; and lung cancer mortality was 1.69 (1.12-2.54) for low vs. high serum levels of L&Z. Adjusting for fruits and vegetables, HRs for all-cancer deaths were 1.44 (95% CI=1.13-1.83) for low vs. high and 1.36 (95% CI=1.15-1.61) for medium vs. high; colorectal cancer mortality was 2.41 (95% CI=1.40-4.18) for medium vs. high, and lung cancer mortality was 1.62 (95% CI=1.07-2.46) low vs. high serum levels of L&Z. Results suggest serum L&Z can reduce risk of cancer mortality. Not only do L&Z reduce carcinogenesis, they also enhance survival among cancer victims. These findings confirm dietary recommendations that promote intake of a variety of fruits and vegetables to protect health and prevent disease. Further studies are needed to explore the physiological mechanisms of this phenomenon.

2673 Metal Oxide Nanoparticles and Bacteria Alter Brush Border Enzyme Activity in an In Vitro Small Intestinal Model
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Models of the gastrointestinal tract (GIT) have gained attention as a useful method for evaluating the digestion and absorption of engineered nanomaterials (ENM) released from food packaging and matrices. The in vitro Caco-2/HT29-MTX intestinal model has been shown to be a high-throughput, cost-effective and relatively fast experimental processes when compared to in vivo models. The human GIT environment, however, is composed of both human cells and the gut microbiota. It is well-known that the intestinal microbiota, which is primarily composed of bacteria, forms a complex ecosystem and plays an important role in the health of the host. The gut microbiota has been shown to be involved in basic human biological processes, including modulating the metabolic phenotype, regulating epithelial development, and influencing innate immunity. The goal of this study was to increase the complexity of the Caco-2/HT29-MTX in vitro model by co-culturing the human cells with two common strains of commensal and opportunistic intestinal bacteria, Lactobacillus rhamnosus GG and a non-pathogenic Escherichia coli strain, respectively. The system was challenged with physiologically relevant doses of pristine or digested zinc oxide (ZnO), iron oxide (Fe3O4), silicon dioxide (SiO2), or titanium dioxide (TiO2) nanoparticles, which are some of the most common ENM added to food and food packaging. To observe and measure the role of bacteria on gut function and to determine how food additives can alter this relationship, we acutely (4 hours) exposed Caco-2/HT29-MTX monolayers cultured with bacteria to metal oxide nanoparticles and analyzed intestinal alkaline phosphatase (iAP), aminopeptidase-N (APN), sucrase isomaltase (SI) and Na+/K+ ATPase activity. Our results showed that following exposure to digested and non-digested ENM, intestinal enzymatic activity was altered. The presence of bacteria may play a protective role, as the presence of bacteria in the model attenuated these effects. Brush border enzymes are known to control many physiological gut processes and activities such as the digestion of peptides and nutrient transport. Changes in their activities may cause chronic disease such as obesity, inflammatory processes and malnutrition.

2674 Comparison of Dietary Exposures to Acrylamide from Intakes of Bread and French Fries to a Proposed New No Significant Risk Level for Acrylamide
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Acrylamide, which formed in foods during frying, baking, or roasting is classified by IARC as a probable human carcinogen (2A) and is on the California (CA) Proposition 65 list of chemicals known to cause cancer or reproductive toxicity. Foods often high in acrylamide include French Fries, cereals, potato chips, breads and baked goods, and also coffee. CA EPA has quantified a no-risk-significant risk level (NSRL) of 0.2 micrograms/day for acrylamide. Under CA Proposition 65, businesses are required to provide a warning if foods contain a listed chemical in amounts that would exceed the NSRL when the foods are consumed (based on mean intakes). The NSRL of 0.2 µg/d was reported to be based on a US EPA oral slope factor of 4.5 mg per mg/kg/d. The US EPA has since revised the oral slope factor to 0.5 mg per mg/kg/d. An NSRL corresponding to this value would be 1.4 micrograms/day (1E-5/(0.5 per mg/kg-d) x 1000 µg/mg x 70kg). While CA EPA has reported that levels of acrylamide in foods are less than amounts believed to cause harmful effects, there have been over 100 notices of violation for acrylamide for potato-based and/or bread products. As a practical exercise, intake modeling was conducted to determine the mean intake of oven-baked fries (=26 g/d), deep-fried fries (=37 g/d) and multigrain bread (=34 g/d). In order to not exceed the NSRL, the concentration of acrylamide in the oven-baked and deep-fried fries must be below 7.7 ppb and 5.4 ppb, respectively, and below 5.9 ppb in bread, based on the mean intake estimates. In the concentration of acrylamide in fries are typically 250 to 450 ppb, and 40 ppb in multigrain bread. While fries are not typically considered a healthy choice, multigrain breads are. If the NSRL were increased based on the new US EPA slope factor, acrylamide concentrations would need to be below 54, 38 and 41 ppb in oven-baked fries, deep-fried fries, and bread, respectively; to not exceed the proposed NSRL. The proposed higher NSRL is unlikely to have any negative implications given that several recent epidemiology studies in large population cohorts have failed to support an association between acrylamide intakes and increased risks of renal cell carcinoma, or of breast, ovarian, endometrial or pancreatic cancer. Based on these findings, it has been suggested that dietary acrylamide was not an important cancer risk factor for humans.

2675 Detecting Genotoxicity within Non-Intentionally Added Substances (NIAS): A Problem Facing the Food Contact Materials Industry
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Alongside intentionally added substances (IAS), safety assessment of non-intentionally added substances (NIAS) in food contact materials (FCMs) is now required under European law. NIAS may include impurities of IAS, or be by-products of the manufacturing process. NIAS are typically assessed by using solvent extractions of FCMs and concentration of the migrates. Assessing genotoxic risk in complex mixtures from FCM migrations poses a significant challenge, further compounded by the unknown identities and low concentrations of NIAS. To determine best approaches for detection of genotoxicity within FCM migrations, a review of Ames, GADD45a assay and carcinogenicity data was conducted for a number of reference genotoxic substances. Using available carcinogenicity data, a ‘permissible daily exposure’ (PDE) with relevance to human daily intake was estimated for each reference substance, along with estimated PDE equivalent concentrations in FCM migrations. These were compared with in vitro assay lowest effective concentrations (LEC) of the proposed TTC limits for safe exposure of NIAS. Comparison of the simulated PDE FCM migration concentrations and in vitro data demonstrated that whilst bioassays do not provide limits of detection equivalent to or below TTC or PDE daily intake values, they can provide useful detection of genotoxic hazard at or below proposed FCM limits of 90 ppb. This study supports the utility of bioassays for the detection of genotoxic hazard of NIAS within FCM migrations.

2676 Mycotoxins and Endotoxin Content Vary between Commercially Available Grain-Based Diets
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Diet choice can affect data interpretation and reproducibility in laboratory animals. Indeed, studies have shown that endotoxins (ET), mycotoxins (MT) and antibiotics in the diet independently affect the development and function of the immune system in rodents, and thus, careful diet selection is critical especially while studying immune-system related disorders. Grain-based (GB) diets typically have higher concentrations of ET compared to purified diets which have very low to undetectable levels. However, it is unknown whether there is lot-to-lot variability. In this study, we analyzed levels of ET, antibiotics and MT in commonly used, commercially available laboratory animal diets (4 GB diets or 3 purified diets). Bioassays were analyzed using the limulus amebocyte lysate assay for ET and biochip array (Randox) customized to simultaneously detect 21 antibiotics and 10 MT from a single diet sample. Antibiotics levels were 2019 SOT Annual Meeting
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Elevated plasma concentrations of ALT, AST and total bilirubin (~20-fold) were found in 1 sample (965 ppb), whereas ocdrotaxin A (0.4 - 1.3 ppb), zearalenone (60 - 162 ppb), deoxynivalenol (0.52 - 1.1 ppm) and fumonisins (56 - 691 ppb) were found in all GB diet samples tested. T2 toxin was detected in 3 out of 4 GB diets (5.6 - 13.8 ppb). Although the levels detected were low, this indicates that some GB ingredients were contaminated by MT known to have toxic and/or immunosuppressive effects. The interactive effects of multiple MT of the 10 most prevalent we measured (–400 others not measured) in vivo cannot be excluded despite their low levels. While certain GB diets may be lower in these toxins than others, we and others have reported the presence and lot-to-lot variability of other contaminants such as mycotoxins, bisphenol A and heavy metals in these diets. We are currently determining whether, as is the case for ET, if there are lot-to-lot differences in MT and antibiotics in GB diets. As anticipated, purified diets are free from such bioactive ingredients, and therefore, provide a clean background to study the toxicological phenotype in animal models.

2677 Assessment of Efficacy of Oroxylum indicum in Rat Model of Thioacetamide-Induced Hepatotoxicity and Its Safety Profile

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Thioacetamide (TAA) is being used as a hepatotoxicant in rat model of thioacetamide induced hepatotoxicity that mimics cirrhosis and hepatic carcinoma in humans. Traditionally stem bark, flowers and leaf of the plant had been used as a remedy for jaundice in India. Whole stem aqueous and ethanolic extracts (500 mg/kg) each were administered by oral gavage in Wistar rats (3 rats/group/sex) post four hours of oral gavage doses of thioacetamide (50 mg/kg, s.c.) in 100 ml/kg/day for 10 days post final dose of TAA. Animals were sacrificed on day 46, serum samples and liver tissues were collected for clinical chemistry and gene expression/antioxidant enzymes/histo-pathology analysis respectively. Clinical chemistry (ALT, AST, LDH, GDH, AFP) and antioxidant enzymes (MDA, CAT, GSH, SOD) results showed statistically significant decreases (p<0.05) with a trend towards recovery. RT-PCR results showed statistically significant reduction (p<0.05) in TNFα, IL6 (Inflammatory cytokines) and NFκB, P38 MAPK (key regulators of inflammation, immunity, apoptosis and wound healing). Histopathology analysis indicated decreased severity and incidence of tumor nodules from severe to mild in aqueous and ethanolic extract treated groups. Acute (OECD 423, 2000 mg/kg) and Subacute (OECD 407, 1000mg/kg) oral gavage toxicity studies did not result in any adverse clinical signs and mortality. Ames assay (up to 500µg/plate, maximum feasible concentration) and chromosomal aberration assay (up to 5000µg/plate) were tested. Negative taken. Further fractionation of plant extract is warranted to explore hepatoprotective activity from a single active fraction. Supported by APT Research Foundation, Pune, India.

2678 Hepatotoxicity of Cannabidiol in the Mouse Model

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Cannabidiol (CBD) is a non-psychoactive ingredient of Cannabis sativa and is a major component of EPIDIOLEX®, a new drug designed for treating epileptic seizures. Utilization of CBD has progressively increased over the last year due to its increased availability to the general public together with aggressive marketing strategies for managing migraine and other pain-associated conditions. Emerging evidence, however, indicates that CBD poses a risk for hepatotoxicity. The goal of this study was to investigate the hepatotoxic potential of CBD delivered with sesame oil to 8-week-old male B6C3F1 mice. Animals were gavaged with allometrically scaled mouse equivalent doses (MED) of either 0, 20, 60, or 200 mg/kg of CBD (acute toxicity, 24 h) or with daily doses of 0.5, 1.5, or 5 mg/kg (chronic toxicity) over 21 days. In both chronic and acute CBD exhibited signs of hepatotoxicity. Specifically, when administered acutely, 200 mg/kg CBD elicited increases in liver-to-body weight (LBW) ratios accompanied by markedly elevated plasma concentrations of ALT, AST and total bilirubin (~20-fold). In the sub-acute study, 50% of mice gavaged with 50 mg/kg CBD exhibited significant toxic manifestations (i.e., tremor and lethargy) on days 3 and 4. Sub-acute CBD exposure was associated with reduced ALT, AST and total bilirubin. Additionally, hepatotoxicity gene expression arrays revealed over 50 genes differentially regulated in the livers of mice gavaged with CBD.

Further analysis highlighted the involvement of genes associated with the oxidative stress response and lipid metabolism as well as a number of cytochrome P450s responsible for drug biotransformation (i.e., CYP1A, CYP2E, CYP2B). In conclusion, mice gavaged with clinically-relevant MED of CBD exhibited classic signatures of cholestatic liver injury. Furthermore, alterations of numerous biochemical pathways associated with lipid and drug metabolism raises serious concerns about the long-term safety of CBD. Support: NIGMS 1P20GM109005 and T32 GM106999; Arkansas Biosciences Institute.

2679 Kalanchoe Plants Traditionally Used for the Treatment of Skin Wounds and Their Antibacterial Activity

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Antibiotic resistance has recently become a matter of public health concern due to the inability of classic antibiotics to keep up with constantly mutating bacteria. Therefore, the discovery and development of novel antibiotic agents able to work against currently resistant bacteria is of the utmost importance. A recent literature revision on the ethnomedical and natural products of the Kalanchoe family, has revealed the diverse biological activities reported for Kalanchoe pinnata. For instance, this succulent plant has been traditionally used for treatment of skin disorders and ear infections in Puerto Rico. However, few research has focused toward the other medicinal species of Kalanchoe plants. In our project, we are currently working with six different species of the Kalanchoe genus. We are very interested in determining the effect of these plants against commonly known bacterial pathogens, especially those present in skin wounds. With this in mind, we used a novel screening method to determine their general antibiotic profile. The Mobile Discovery Kit was used to test their activity against common bacteria found in human saliva. A small sample of the leaves were collected, cleaned and cut to perform the assay. After 24 hrs of inoculation, all six species showed notable to strong antimicrobial activity. Positive and negative controls were included in the screening. Five out of six samples (83%) showed no bacterial growth. The results suggest that the antibiotic profile of these species is not controlled by their potential as source of antibiotics may be indeed great. Polar extracts (methanol and aqueous) from the six species were prepared. Their testing against bacteria typically found in skin wounds, are currently in progress and will be presented. As part of a long term goal, these extracts will be purified and characterized to identify the biologically active agents responsible for the described antibacterial effect. This will allow for a better understanding of the Kalanchoe family potential and future applications for the development of new antimicrobial agents.

2680 Cellular Accumulation of Cocaine Following Osteoblastic Differentiation in MC3T3-E1 Cells


Cocaine is one of the most widely abused drugs. It has been reported that the chronic exposure to cocaine can lead to an adverse effect on skeletal development and caused to accumulation of cocaine and its metabolites in bone in rat experiment. However, the accumulation mechanisms in the bone have not been well documented. MC3T3-E1 cells, a mouse calvaria osteblast-like cell line, can induce osteoblastic differentiation and mineralization by treatments with beta-glycerophosphate (GP) and ascorbic acid. In this study, we investigated the effect of cocaine on cellular proliferation and the changes of accumulation of cocaine and its main metabolite, benzoylecgonine (BE), following differentiation using MC3T3-E1 cells. To examine cell proliferation, cells were exposed to 0-1 mM cocaine and cultured for 2 days. The cell viability assay indicated that cocaine promotes cell proliferation. At the concentration of 500 µM, the viable cell counts were incremented to 1.4 times. Furthermore, to examine whether cellular accumulations of cocaine and BE are changed by differentiation, we compared their cellular concentrations between with and without mineralization. The cells were cultured with or without both 10 mM GP and ascorbic acid in the presence of 0-100 µM cocaine for 10 days. After the culture, ALP activity, a marker of osteoblastic differentiation, and CA deposition were increased with treatments of GP and ascorbic acid, suggesting that the cells were differentiated under this culture condition. LC/MS/MS analyses revealed that concentrations of cocaine and BE increased exposure-dose-dependently. The concentration of cocaine in differentiated osteoblasts was significantly higher than that in undifferentiated osteoblasts.
However, concentration of BE was not affected by differentiation. These results indicate that chronic exposure to cocaine might have an adverse effect on bone development.

2681 Protective Effect of Ethanol Leaf Extract of Vitellaria paradoxa (Gaertn F) on Sodium Arsenite-Induced Toxicities in Male Wistar Rats


Globally, the inadvertent exposure to arsenic has been associated with diverse diseases including cancers, ulceration, neurological and reproductive disorders. Researches on arsenic toxicity are therefore being directed towards reducing exposure and/or intercepting its activities. In this regard, the potency of ethanol leaf extract of Vitellaria paradoxa (ELVpar) medicinal plant with antiadictive and antiproliferative properties used in treatment of entic infections, wounds, leprosy was assessed on sodium arsenite (SA) - induced toxicities in rats after ethical approval (Reference: UI-ACUREC 16/0019). Experimental rats were divided into eight groups of five animals each. Group 1 was given distilled water. Group 2: SA at 2.5 mg/kg, Group 3: vitamin E 100 mg/kg, Group 4: SA + vitamin E while Groups 5 - 8 received extracts alone or SA + extract at 100mg/kg and 200mg/kg respectively. After two weeks of treatments, the animals were sacrificed by cervical dislocation. Treatment with SA alone caused significant increase (p<0.05) in serum ALT, ALP activities, Urea and creatinine and concentration of uric acid compared with its control. When co-administered with ELVpar a significant reduction of approximately 1.3 (ALT), 2.6 (ALP), 2.2 and 1.5 (urea and creatinine) folds respectively and 1.6 (MDA) folds respectively was observed. This result was corroborated with observations from histological analysis of the liver and kidney. In addition, treatment with SA led to mild expression of BCL-2 protein > NF-Kb = p53 in kidney as compared with the control. Activation of nuclear factor-Kb (NF-Kb) a transcription factor that promotes cell proliferation and suppresses apoptosis is most likely due to production of reactive oxygen species by the toxicant. Co-administration of ELVpar increased the expression of p53 and decreased the BCL-2 protein expression. It was concluded that ELVpar have hepatoprotective, nephroprotective and apoptotic properties against sodium arsenite-induced toxicities.

2682 Evaluating the Effects of Essential Oils on Steroidogenesis in a Human Feto-placental Co-Culture System

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We determined whether 5 common essential oils (EOs), basil, fennel seed, orange, black pepper and sage, interfered with feto-placental steroidogenesis in a co-culture model composed of human fetal-like adrenocortical H295R and trophoblast-like BeWo cells. After a 24h exposure of the co-culture to EO concentrations ranging from 0.005% to 0.005%, only basil and fennel seed oil had effects, significantly increasing production of estradiol, estrone, dehydroepiandrosterone, androstenedione, progesterone and estriol, but not testosterone. Using real-time quantitative RT-PCR, basil and fennel seed oil were shown to significantly alter the expression of several key steroidogenic enzymes, including CYP11A1, 3β-HSD1/2, SULT2A1, and 17β-HSD1, -4, and -5 as well as cholesterol transporter STAR. Particular attention was placed on the promoter-specific expression of aromatase (CYP19), the key estrogen biosynthetic enzyme essential for healthy pregnancy. In the co-culture, basil and fennel seed oil stimulated placental-specific promoter I-1 derived CYP19 expression in BeWo cells, whereas in the H295R cells, there was stimulation of promoter-II specific expression of CYP19. CYP19 catalytic activity, determined by titrated water-release assay, was significantly increased by basil and fennel seed oil in both cell types in co-culture, which correlated with increased mRNA levels. Furthermore, the isomers estragole and trans-anethole, the most abundant chemical constituents of basil and fennel seed oil, respectively, were found to have almost identical effects in the co-culture to those of the complete essential oils. Our results indicate that further study is necessary to determine the potential risks of using basil and fennel seed oils during pregnancy, considering their potential to disrupt steroidogenic enzyme activity and expression in vitro. Investigations into the mechanisms by which basil and fennel seed oil increase STAR expression in the H295R cells in co-culture are currently underway, focusing on the role of increased progesterone production. In BeWo cells in co-culture, STAR expression was significantly decreased by basil and fennel seed oil. As placental cells do not translate STAR into protein, we are studying effects on MLN64, a closely related gene that is suggested to replace the function of STAR protein. Effects of EOs and main chemical constituents on signaling pathways involved in the promoter-specific regulation of CYP19 are also under investigation.

2683 Halogenated Marine Natural Products and Human Health: Exploring the Activity of Halogenated Methyl Bipyroles as Agonists for the Aryl Hydrocarbon Receptor

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The presence of anthropogenic halogenated aromatic hydrocarbons (AHAs) in the marine environment and their potential impacts on human health through consumption of contaminated seafood have been a concern for half a century. Synthetic AHAs such as polychlorinated biphenyls (PCBs) and polychlorinated diphenyl ethers (PBDEs) are ubiquitous in the environment and their toxicity in animal systems is well known. More recently, a variety of naturally occurring, bioaccumulative AHAs have been identified in the oceans, including in upper-trophic-level animals and, increasingly, in seafood. These halogenated marine natural products (HMNPs) include methylxoylated PBDEs (MeO-PBDEs), halogenated methyl-2,1'-bipyroles (MBPs), and halogenated N,N-dimethyl-2,2'-bipyroles (DMBPs). Many of the HMNPs are structurally similar to anthropogenic AHAs, but their potential toxicity and impacts on human health are poorly understood. Here, we show that some of these compounds are able to bind to and activate the aryl hydrocarbon receptor (AhR), through which many of the synthetic AHAs act to cause toxicity. Several MBPs and DMBPs induced CYP1A1 in human HepG2 cells, although with potencies and efficacies less than those of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), whereas two natural MeO-PBDEs were less active. The MBPs and DMBPs also induced Cyp1a1 in zebrafish larvae in vivo. The results highlight the need to better understand the environmental behavior, biological activity, and potential human health impacts of HMNPs. Supported by P01ES021923, OCE-1314642, P01-ES021921, OCE-1313747, and Sea Grant NA19OAR4170104.

2684 Evaluation of Toxic Effects of Methanolic Leaf Extract of Spigelia anthelina on Wistar Albino Rats


Spigelia anthelina Linn also known as pinkroot is a flowering plant in the family of Loganiaceae. It is a tropical annual weed that grows in every part of Nigeria but used as medicinal plant in South Western part of the country to treat chronic hemelnosis in both humans and animals. The plant has been reported for its use as a laxative, antibacteria, and for the treatment of tumors, cancer, HIV, headache, throbbing pain, common cold, heart diseases, chest pain, as vermifuge and to reduce migraines. It is also used alone or in combination with other plants for toothache, foul mouth odour and fever. The plant is popular for its use as worm expellant or to destroy tapeworm and round worms in the body. Spigelia anthelina is used for chronic catarrh, difficulty in breathing and fierce palpitations. It is also an important remedy in pericarditis and other diseases of the heart. Good as this plant is, some people are afraid of its probable toxic effect on which scanty information is available. This then prompts us to study the toxic effect of its leaf extract. The methanolic leaf extract of the plant was administered per os at 200, 400, and 800 mg/kg to three groups A, B and C of Wistar albino rats respectively for 28 days while the group D which served as control was administered also per os with 3ml/kg of distilled water. The obtained blood samples from anaesthetized rats were then analyzed for hematolgy, serum biochemistry and semen. The results obtained showed no significant effect (p>0.05) on the blood parameters, but significant increase (p<0.05) was observed for alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase for the groups administered with 400 and 800 mg/kg compared with the control. There was also a significant decrease (p<0.05) in the sperm concentration of the rats administered with 800 mg/kg of the extract. It was then concluded that the plant could show some toxic effects at high doses and therefore care must be taken when it's been used at high doses.
2685 Investigating the Effects of Chia Seed Consumption on Phase II Detoxification Enzymes and Lipid Peroxidation in Rat Liver


The demand and consumption of functional food has grown significantly worldwide in recent years as people become more health conscious. Phytochemicals are non-nutrient compounds that can be attained from fruits, vegetables, and grains. Consumption of phytochemicals has been correlated with a reduced risk of certain chronic illnesses; these include cardiovascular diseases, obesity, diabetes, and cancer. As such, use of chia seeds (Salvia hispanica L.) has significantly increased over the years, although it has been a common traditional food in many South and Central American countries for centuries. Chia seeds are a rich source of essential fatty acids—such as ω-3 alphalinolenic acid and ω-6 linoleic acid—which are vital for human health. Although chia seeds are mostly consumed for their rich nutritional value, they may exert benefits beyond their nutritional source due to the phytochemicals they contain. Chia seeds are roughly 9% phenolic and include phytochemicals such as myricetin, quercetin, kaemferol, caffeic acid, flavonol glycosides, and chlorogenic acid among others. In this study, we investigated the levels of Phase-II enzymes, GSH content, and lipid peroxidation in rat livers, while varying the amount of chia seeds in the diet of the subjects. The rats (Sprague Dawley) were fed 1.0%, 2.2% or 4.4% chia seeds/kg body weight for 4 months. The levels of GSH, Glutathione S-Transferase (GST), NADPH Quinone Oxidoreductase (NQO), and Thioctic Acid Reactive Substances (TBARS) levels were determined in liver. To the best of our knowledge, no study measuring the effects of chia seeds on detoxification or antioxidant enzymes is conducted. Our results show that GSH levels were dependent to increasing chia seed intakes. Chia seed supplementation in diet does not seem to affect the levels of GST, whereas the levels of NQO seem to significantly increase with higher doses. These results suggest that the induction in GSH and QR levels by chia seeds may provide substantial protection against toxicities and thus be beneficial to human health. Also, the levels of TBARS remain unchanged in both control and chia seed-fed animals suggesting that the supplement of chia seeds in the diet may not cause oxidative damage in liver tissues.

2686 The In Vitro Antibacterial Activity and Safety of Morinda lucida Leaf Extracts against Salmonella Serovars Relevant in Livestock Infections

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Salmonella infections are of great importance in human and animal health. They cause significant morbidity and mortality worldwide. Infections caused by non-typhoidal Salmonella are either non-invasive, self-limiting enteric infections or invasive systemic infections that require effective antimicrobial therapy. The development of resistant strains has reduced the efficacy of the conventional antibiotics. Therefore, there is an urgent need to develop drug leads or templates with good activity against these pathogens. Morinda lucida is a native tree species of Africa that has been used extensively in African traditional medicine for the treatment of symptoms similar to human typhoid and malaria fever. The aim of this study was to investigate the antibacterial activity of the leaf extracts of eight Salmonella serovars. Acetone and aqueous leaf extracts of Morinda lucida were screened for antibacterial activity against several serovars of Salmonella enterica subsp. enterica including S. enterica serovar Gallinarum (birds), Dublin (birds and ruminants), Choleraesuis (pigs), Braenderup (birds), Ildken (humans and birds), Kottbus (birds), Typhimurium (birds and ruminants) and Enteritidis (birds and humans) using a serial microdilution assay. The cytotoxic potential of the acetone and aqueous extracts against human colon cancer (Caco-2) cells was also determined. The minimum inhibitory concentration (MIC) of the selected plant extracts generally ranged from 0.09 to 1.87 mg/ml with the acetone extract having the best activity against all the tested strains. The levels of positive control, gentamycin, against the tested strains ranged from 0.0002 to 0.01 mg/ml. The LC50 values of the acetone and aqueous extracts against the Caco-2 cells were 0.46 and 0.33 mg/ml respectively. The selectivity index (SI) values of the acetone extract ranged from 1.00 to 6.57 and SI of the aqueous extract ranged from 2.3 to 8.28 indicating that the observed antimicrobial activity was generally not due to toxicity to mammalian cells. The results obtained validate in part the potential usefulness of this plant species as an alternative for treatment of human and animal salmonellosis. However, in vivo data is necessary to further investigate this claim.

2687 Inhibition of Pro-hypertensive Soluble Epoxide Hydrolase (sEH) Activity by Magnolia Bark Extract in a One-Step Inhibition 14,15-DHET ELISA


Soluble epoxide hydrolase (sEH) biotransforms anti-hypertensive epoxyeicosatrienoic acids (EETs) to dihydroxyeicosatrienoic acids (DHETs). Increased sEH activity in kidney, heart and brain cells, which lowers EET levels, has been linked to hypertension, cardiovascular diseases and stroke, respectively. Inhibition of sEH-dependent conversion of 14,15-EET to 14,15-DHET, a major metabolite of sEH, has been evaluated as an approach for the prevention and treatment of hypertension and stroke. The aim of this study is to find whether a Chinese medicine, magnolia bark extract (FDA GRAS food additive), inhibits activity of pro-hypertensive sEH. Previously inhibition of sEH activity has been measured by a two-step inhibition assay consisting of a 30 min incubation step with the sEH enzyme, 14,15-EET and an inhibitor followed by ethyl acetate extraction and quantification of the 14,15-DHET level by ELISA. Using a novel one-step inhibition ELISA developed by combining the inhibition and quantification steps, sEH activity inhibition assays using 12-[(tritylcyclo[3.3.13.7]dec-1-ylamino)carbonyl]alaminododecanoic acid (AUDA, positive control), magnolia bark extract, honokiol (major constituent of magnolia bark), carrot and watermelon juices and vitamin B complex were carried out. A reaction mixture (100 µl) of 80 nM EET, 15.6 nm sEH enzyme and with or without a dietary supplement was pre-incubated for 30 min in 14,15-DHET antibody-coated wells of the 96-well plate to capture 14,15-DHET formed from EET. The levels of 14,15-DHET formation and % inhibition by the dietary supplement were quantified using 14,15-DHET standards after addition of 14,15-DHET-conjugated HRP (100 µl) to the standard or the reaction mixture (100 µl) in each well. The AUDA was a potent inhibitor of the sEH activity. Carrot and watermelon juices and vitamin B complex did not inhibit sEH activity, whereas 4 mg/ml and 10 mg/ml magnolia bark extract inhibited sEH activity by 83% and 96%, respectively, and 1 mM honokiol inhibited the sEH activity by 80%. Honokiol, a bioactive component of magnolia bark, added to human kidney ACHN cell culture media inhibited cytosolic sEH activity. Thus, consumption of magnolia bark extract might lower cellular sEH activity and ameliorate hypertension and heart-related diseases.

2688 Characterization and Formulation of a Black Cohosh Root Extract (BCE) Lot to Be Used in Rodent Toxicology Studies

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Black cohosh (Actaea racemosa) is sold as a dietary supplement for the treatment of menstrual and menopausal symptoms in women. Although it has widespread human exposure, there is limited safety data in rodents and humans. The National Toxicology Program (NTP) is investigating the toxicity of BCE in rodents. The objective of this work was to screen commercially available lots sold as BCE to select an unformulated product for use in NTP studies. Multiple lots of unformulated and formulated products sold as BCE, standard reference materials of BCE and potential adulterants (Chinese, red, and yellow cohosh) were analyzed using a combination of non-targeted and targeted analytical techniques. Authenticity was confirmed by chemical fingerprinting and DNA barcoding. Based on the results, a lot was selected for further characterization. The constituents of the selected lot (actein, 0.47%; 27-deoxyactein, 2.09%; ferulic acid, 0.04%; isoflavonic acid, 0.70%; caffeic acid, 0.28%; cimicifugoside C, 2.13%; 26-deoxyisofagomine, 0.08%; magnoflorine, 0.01%) were determined by LC-MS-MS. Other analyses included moisture content (5.6%), ash (5.8%), and nutritional content (fat, carbohydrate and protein). The lot was also analyzed for contaminants (heavy metals, ≤476ppb; pesticides, ≤0.3ppm; mycotoxins, ≤50ppb; microbial content, ≤10 CFU/g). A method for formulation of BCE in 0.5% methylcellulose was developed and a formulation analysis method was validated to quantify isoflavonic acid. Analytical method was linear (r ≥0.99) and accurate and homogeneous (% relative error, RE ≤ ±10 %). The formulations were stable for multiple markers (actein, 27-deoxyactein, isoflavonic acid and cimicifugoside C) up to 42 days with % RE ≤ ±20 of day 0. These data demonstrate that the BCE lot is suitable and can be formulated to be used in rodent toxicological studies.

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2689 Inhibition of Vascular Endothelial Growth Factor-Induced Angiogenic Effects in Human Umbilical Endothelial Cells by Cinnamon Extract

It remains elusive how safe the use of Japanese herbal medicines is during pregnancy and if the herbal medicines do any harm to pregnancy and placentation. Since components of herbal medicines are more complicated than those of modern medicines, the former’s actions may be more complicated than the latter’s. Long-term fetal exposure to herbal medicine may, therefore, adversely affect fetal or postnatal development. Hazard assessment of traditional Japanese herbal medicine for pregnant women is important as herbal medicines are commonly perceived to have fewer side effects than modern medicines. Angiogenesis actively undergoes throughout pregnancy, and the dysplasia of corpus luteum and placenta due to vulnerable angiogenesis results in miscarriage or low birth weight. The present study investigated the effect of cinnamon extract, which is often taken during gestation period as a component of Goreisan or Kakkonito, on the Vascular Endothelial Growth Factor (VEGF)-induced tube formation in vascular epithelial cells. Methods: Human Umbilical Vein Endothelial Cells (HUVECs) were cultured on Matrigel-coated plate and treated with VEGF at 20 ng/mL in absence or presence of cinnamon extract for 20 h. As treatment with cinnamon extract at 50 μg/mL inhibited VEGF-induced tube formation in HUVECs, cinnamon extract was separated into three fractions, chloroform fraction (ChrF), butanol fraction (ButF) and water soluble fraction (WatF), and the effect of each fraction on tube formation in HUVECs was similarly investigated. Results: Of the three separated fractions, ChrF showed an inhibitory effect on tube formation. We separated components of ChrF using column chromatography and identified cinnamic acid as a major component of ChrF using Nuclear Magnetic Resonance (NMR) and mass spectrometry. Cinnamic acid was quantified using thin layer chromatography. The content of cinnamic acid in cinnamon extract was 1.70%. Tendency to inhibit effect on tube formation was similarly observed after treatment with cinnamic acid at 5.7 μM, corresponding to cinnamon extract at 50 μg/mL. The results show that cinnamic acid might be an active principle of cinnamon extract for suppression of VEGF-induced tube formation in HUVEC.

2690 Development and Validation of an Analytical Method for Quantitation of Alpha-Pinene in Rodent Blood by Headspace GC-MS
M. A. Silinski1, J. C. Blake1, J. Licause1, R. A. Fernando1, V. G. Robinson2, and S. Waidyanatha1. 1RTI International, Research Triangle Park, NC, and 2NIEHS/NTP, Research Triangle Park, NC.

Alpha-pinene (AP), produced by pine trees and other plants, is the main component of turpentine and is used as a fragrance and flavor ingredient. Exposure to AP occurs via use of personal care and household cleaning products and in the lumber industry. Despite widespread exposure, the toxicity data for AP are limited. The objective of this work was to develop and validate a method to quantitate AP in rat and mouse mammary tissue, a potential target tissue, in support of the National Toxicology Program toxicokinetic and toxicology studies. Standards were prepared by spiking a ~100 mg aliquot of mammary tissue with 100 μL of spiking solution containing AP and internal standard (IS; AP-d3) in 50/50 ethanol/saline in a 2-mL headspace vial. The vial was sealed with a metal crimp-top cap, equilibrated for 10 min at 60°C, and a 500 μL headspace sample was analyzed by GC-MS using single ion monitoring ([M+H]+ m/z 136 (AP) and 139 (IS)). A DB-5MS column was used with oven temperature ramped from 40°C to 150°C in 9 min. The method was successfully validated in female Sprague Dawley rat mammary tissue over the concentration range 100-5000 ng/g. Matrix standard curves were linear (r ≥ 0.99), and the percent relative error (%RE) values were ≤ ±12% for standards at all levels. Small background peaks were detected in the matrix and method blanks, but the response was low and did not interfere with method performance. Absolute recovery was low (2%) likely due to high lipophilicity of AP. However, the limit of detection, determined from the standard deviation at the lower limit of quantitation (100 ng/g), was 17.7 ng/g, demonstrating adequate sensitivity. Recovery levels were 90% at 900 ng/g for intra- and inter-day precision (% relative standard deviation, RSD) and accuracy (%RE) were ≤5% and ≤7%, respectively, for quality control standards prepared at 250 and 2500 ng/g. Standards as high as 20,000 ng/g could be analyzed using a lower injection volume (20 μL) or by extrapolating the calibration curve beyond 5000 ng/g, with mean %RE ≤ ±1.4% and %RSD ≤3%. Smaller sample sizes (~50 ng) could also be analyzed, with mean %RE ≤ 2.2% and %RSD ≤ 2%. The method was validated for female Harlan Sprague Dawley rat and B6C3F1 mouse mammary tissues; %RE values were ≤ ±5% and %RSD ≤ 2%. These data demonstrate that the method is suitable for the analysis of AP in rodent mammary tissues generated from toxicokinetic and toxicology studies.

2691 Development and Validation of an Analytical Method for Quantitation of Alpha-Pinene in Rodent Mammary Tissue by Headspace GC-MS
M. A. Silinski1, J. Licause1, T. Venoyama1, J. C. Blake1, R. A. Fernando1, V. G. Robinson2, and S. Waidyanatha1. 1RTI International, Research Triangle Park, NC, and 2NIEHS/NTP, Research Triangle Park, NC.

Alpha-pinene (AP), produced by pine trees and other plants, is the main component of turpentine and is used as a fragrance and flavor ingredient. Exposure to AP occurs via use of personal care and household cleaning products and in the lumber industry. Despite widespread exposure, the toxicity data for AP are limited. The objective of this work was to develop and validate a method to quantitate AP in rat and mouse mammary tissue, a potential target tissue, in support of the National Toxicology Program toxicokinetic and toxicology studies. Standards were prepared by spiking a ~100 mg aliquot of mammary tissue with 100 μL of spiking solution containing AP and internal standard (IS; AP-d3) in 50/50 ethanol/saline in a 2-mL headspace vial containing 18 stainless steel beads. The vial was sealed and homogenized for 30 sec for 2 cycles at 1000 rpm. Each vial was equilibrated for 10 min at 60°C and a 200 μL headspace sample was analyzed by GC-MS using single ion monitoring ([M+H]+ m/z 136 (AP) and 139 (IS)). A DB-5MS column was used with oven temperature ramped from 40°C to 150°C in 9 min. The method was successfully validated in female Sprague Dawley rat mammary tissue over the concentration range 100-5000 ng/g. Matrix standard curves were linear (r ≥ 0.99), and the percent relative error (%RE) values were ≤ ±12% for standards at all levels. Small background peaks were detected in the matrix and method blanks, but the response was low and did not interfere with method performance. Absolute recovery was low (2%) likely due to high lipophilicity of AP. However, the limit of detection, determined from the standard deviation at the lower limit of quantitation (100 ng/g), was 17.7 ng/g, demonstrating adequate sensitivity. Recovery levels were 90% at 900 ng/g for intra- and inter-day precision (% relative standard deviation, RSD) and accuracy (%RE) were ≤5% and ≤7%, respectively, for quality control standards prepared at 250 and 2500 ng/g. Standards as high as 20,000 ng/g could be analyzed using a lower injection volume (20 μL) or by extrapolating the calibration curve beyond 5000 ng/g, with mean %RE ≤ ±1.4% and %RSD ≤3%. Smaller sample sizes (~50 ng) could also be analyzed, with mean %RE ≤ 2.2% and %RSD ≤ 2%. The method was validated for female Harlan Sprague Dawley rat and B6C3F1 mouse mammary tissues; %RE values were ≤ ±5% and %RSD ≤ 2%. These data demonstrate that the method is suitable for the analysis of AP in rodent mammary tissues generated from toxicokinetic and toxicology studies.

2692 hTERT Immortalized Adult Dermal Melanocytes: An In Vitro Cell Model for the Study of Skin Pigmentation
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Skin pigmentation is a complex process; melanocytes produce melanin and package it into melanosomes that are in turn exocytosed into the surrounding extracellular matrix. Numerous genes play roles in controlling pigmentation at various levels of melanin production. Mutations in these genes are characteristic of multiple skin disorders, including hyperpigmentation, hypopigmentation, and mixed hyper-/hypopigmentation. Additionally, extrinsic factors secreted by the surrounding resident cell types also regulate the melanin expression in adult melanocytes. Human primary cells can be a useful model for elucidating melanocyte biology. However, primary cells have their limitations such as donor variability and limited lifespan. Consequently, a need exists for a more robust human cell model system for the study of skin pigmentation. In this study, we immortalized primary dermal melanocytes by expressing human telomerase reverse transcriptase (hTERT) in cells that were isolated from an adult donor. The immortalized primary melanocytes were cultured continuously for more than 40 population doublings without any signs of replicative senescence, yet retained melanin production. The immortalized primary melanocytes maintained a consistent expression of the melanocyte-specific marker TRP-1, and lacked expression of the fibroblast-specific marker TET7. In addition, we demonstrate the capability of these immortalized primary melanocytes to transfer melanosomes to keratinocytes, the ability to modulate melanogenesis with stimulators and inhibitors, and their capacity to incorporate into a functional 3D human dermal organotypic culture. Taken together, the hTERT immortalized melanocytes provide a versatile in vitro cell model for the study of melanin production and melanocyte:keratinocyte interactions in the dermal environment.
In vitro three-dimensional skin tissue models have become the state-of-the-art approach to studying human skin physiology and pathology with the ultimate goal to reduce and replace animal-based toxicological assessment of chemicals. Any reactive agent must first penetrate the skin’s barrier to elicit a reaction in the epidermis and dermis. Thus, understanding the functional composition of the skin’s barrier is a crucial step in understanding how chemicals can potentially gain access into the skin and subsequently influences all downstream reactions. The lipid composition of both the Phenion Full-Thickness (FT) Skin Model epidermis and the Phenion Open Source Reconstructed Epidermis (OS-Rep) was analyzed with thin-layer chromatography. When analyzed simultaneously, the lipid profiles of both skin models closely matched the profile of native human foreskin epidermis. Ceramides, cholesterol, cholesterol derivatives, triglycerides and phosphatidylcholine were identified in all samples tested. Immunofluorescence analysis of keratinocytes isolated from skin biopsies and the epidermis of both the FT and OS-Rep models revealed the presence of major enzymes found in epidermal lipid metabolism, (ceramidases; serine palmitoyltransferase). When comparing to intact human skin, these 3D models presented with similar lipid patterns and key barrier enzymes involved with human skin lipid synthesis. Taken together this provides strong evidence that the physiological barrier and function of the models, mainly located in the stratum corneum, are compatible with human skin. This confirmation was required prior to conducting substance testing in these skin models, (in vitro skin irritation; penetration studies). Only a functioning and selective barrier can discriminate between chemicals which remain on the tissue surface or those that can permeate the skin and subsequently influence the skin’s vascular systems. Thus, for experiments which require a barrier function both the FT and the OS-Rep models are well-suited as in vitro surrogates for native human skin, or epidermis, respectively.

The aim of this study was to correlate the lipid composition of native human foreskin epidermis and different in vitro skin models, including two 3D models (Phenion Full-Thickness (FT) Skin Model and Phenion Open Source Reconstructed Epidermis (OS-Rep)) with the corresponding lipid composition of human foreskin epidermis. The lipid composition of the 3D models was analyzed by thin-layer chromatography. The results showed that the lipid profile of the 3D models closely matched that of human foreskin epidermis, indicating that these models are suitable for studying skin physiology and pathology.

**Development of new methods to qualify the integrity and the barrier function of the skin is necessary before effecting ex vivo skin toxicity tests in reliable conditions. To avoid unsuitable over-prediction of the dermal absorption by the use of impaired skin preparations, the OECD guidelines 428 and 430 require skin integrity check using reference compounds like caffeine for measurement of transcutaneous passage and of transepithelial electrical resistance (TEER) respectively. Caffeine transcutaneous passage is usually performed to qualify dermal delivery, such as that through the stratum corneum, that is tested with human skin. This confirmation was required prior to conducting substance testing in these skin models, (in vitro skin irritation; penetration studies). Only a functioning and selective barrier can discriminate between chemicals which remain on the tissue surface or those that can permeate the skin and subsequently influence the skin’s vascular systems. Thus, for experiments which require a barrier function both the FT and the OS-Rep models are well-suited as in vitro surrogates for native human skin, or epidermis, respectively.
2697 Dermal Toxicology Application Area Changes as Compared to Theoretical Total Surface Area of Hanford Miniature Swine over 18 Weeks

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Dermal toxicity studies are frequently initiated in growing animal models. The test compound is generally applied on a Dermal Application Area (DAA) that is frequently set at certain ratio to total surface area. Since the growth during juvenile period can be significant; the DAA is likely to be impacted during the course of a chronic study. The purpose of this study was to determine the relative proportion of the DAA to Total Body Surface Area (TBSA) will change proportionally over time with the growth of the miniature swine, and 2) to compare three TBSA formulas. The ratio DAA (cm² surface area) to TBSA was calculated from body weight at periodic intervals for 18 weeks in 16 male and 16 female Hanford miniature swine (~4 mo old Hanford miniature swine, averaging ~14 kg at initiation). Two mid-back 5 cm x 5 cm (25 cm²) DAA's, one located on each side of the spine, were used on each subject. The TBSA (m²) was calculated by the well-recognized Spector formula (9.5 x BW(G)0.3710000) as well as the Brodie and Wachtel formulas. Using the Spector formula, the mean ratio DAA to TBSA for week 0 and 8 (N = 32) for an application area was 0.46% ± 0.04 and 0.51% ± 0.06 (MEAN ± SD), respectively. After week 8, subsequent periodic measurements of the ratio of TBSA to DAA remained steady suggesting proportional changes in growth of both DAA and TBSA. The correlation of the three formula ratios was >0.99. Thus, comparable TBSA and DAA/TBSA ratio between the three formulas suggested the well-recognized Spector Method is a valid choice.

2698 Skin Penetration of [3H]Octanoic and [14C]Decanoic Acids after Topical Application of C8910 Formulations to Excised Pig and Human Skin

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C8910 is a biopesticide consisting of octanoic, nonanoic, and decanoic acids. The objective of this study was to determine the skin disposition (evaporation, skin surface residue and penetration) of two of the fatty acids after topical application of aqueous dilution of three different emulsifiable concentrates in a test system predictive of skin absorption in vivo. Formulations were spiked with tritium labeled octanoic acid and carbon-14 labeled decanoic acid and applied to excised pig or human skin. Data, expressed as percent of applied dose, were statistically analyzed (p = 0.05). Comparing the 3 concentrate dilutions, there were no significant differences in evaporation (approximately 60% for octanoic acid and 40% for decanoic acid), percutaneous penetration (about 20% for octanoic acid and 15% for decanoic acid) and residue remaining on the skin surface (approximately 5% for octanoic acid and 20% for decanoic acid) at 24 hours. These results were compared to a mineral oil formulation previously registered with the US EPA for livestock fly control. While there were no significant differences in evaporation, penetration was significantly higher and skin surface residue significantly lower for the mineral oil formulation. Since the pesticidal activity of the fatty acids is influenced by skin penetration, these results support their formulation in emulsifiable concentrates for livestock fly control.

2699 Solute Partitioning from Formulations to Build Skin LFER Models

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Permeation of chemical solutes through skin can create major health issues especially to workers in the metalworking industry. Using the membrane-coated fiber (MCF) as a solid phase membrane extraction approach to simulate skin permeation, we utilized partition coefficients for 37 solutes under 90 treatment combinations that could broadly represent formulations that could be associated with occupational skin exposure. These formulations were designed to mimic fluids in the metalworking process, and include: one of mineral oil, polyethylene glycol-200, soluble oil, synthetic oil, or semi-synthetic oil; at a concentration of 0.05 or 0.5 or 5 percent; with solute concentration of 0.01, 0.05, 0.1, 0.5, 1 or 5 ppm. Linear free-energy relationship (LFER) models were then developed from these data sets to estimate the effect of these multiple variables on skin partitioning. Partition coefficients range from 0.015 to 1279 (-1.820 to 3.107 on the base 10 logarithm scale) and it was somewhat surprising that the smallest partition coefficients were associated with higher concentrations of solute present in the formulation. The improvements in r², Adj-r², Q₁₀₀₀, and Q₁₀₀₀₀ (LOSO = leave one solute out) for the LFERs were quite noticeable. The single LFER model was shown to be inadequate (Q₁₀₀₀₀ = 0.57), but extensions that account for experimental conditions provide important improvements in estimating solute partitioning from selected formulations into the MCF. The benefit of the Expanded Nested-Solute Concentration LFER model (Q₁₀₀₀₀ = 0.88) then compared to the Crossed-Factors LFER model (Q₁₀₀₀₀ = 0.68) was only revealed through a careful LOSO cross-validation that properly addresses the existence of replicates to avoid an overly optimistic view of predictive power. Finally, the partition theory that accompanies the MCF approach is thoroughly tested and found not to be supported under sufficient experimental occupational exposure in the metalworking industry. Our resulting model provides interpretations that are useful for identifying solutes whose chemical structures are consistent with low predicted levels of dermal permeability.

2700 Large Data Sets and Quantitative Risk Assessment (QRA) for Dermal Allergens


The use of quantitative risk assessment (QRA) principles to evaluate the skin sensitization potential of ingredients in consumer products has seen increasing use and development. A key outcome of the QRA is identifying a concentration where the induction of sensitization is not expected in humans, the No Expected Sensitization Level (NESIL). This NESIL is translated to an Acceptable Exposure Level (AEL) for consumers by dividing the NESIL by Safety Assessment Factors (SAFs). These SAFs account for uncertainties in extrapolating from the experimental conditions used to derive the NESIL to a realistic in-use exposure. To address this gap, we utilized data from a Human Repeated Insult Patch Test (HRPT) in a simple vehicle. However, historical databases of HRPTs are often available within consumer product companies on multiple product formulations that contain the same ingredient, in aggregate these datasets can be large encompassing 100s - 1000s of tests. This presents a unique opportunity to provide additional confidence in establishing safe ingredient use levels based on metrics for presence and percent composition of ingredients. Clusters of comparable formulas were identified from this map using graph modularity. These clusters were evaluated to confirm safe use levels and determine the need to apply SAFs across different formula types. For example, citral was included in 44 cosmetic formulas in 3 distinct clusters and no sensitization was observed up to the maximum tested concentration of 0.01%. The clusters were observed to be consistent, members of each cluster contained only similar formula backbones. This consistency confirms the approach to use modularity to identify formula clusters in this map. The separation of the clusters demonstrate that consideration should be given to adequate formulation based SAFs when data is read across between clusters. Using large historical HRPT data sets and QRA shows promise as an approach for cosmetic safety guidance.

2701 Experimental Induction of Allergic Contact Dermatitis in Sinclair, Hanford, and Yucatan Minipigs

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The objectives of this study were to induce and characterize allergic contact dermatitis using 2,4-dinitrofluorobenzene (DNFB) in Sinclair, Yucatan, and Hanford minipigs, and assess the efficacy of corticosteroids in treatment of dermatitis (N = 3 per breed, all males). During acclimation the entire dorsum of the minipig was shaved, washed, and topical dermal challenge sites were marked on the dorsum of each animal. Sensitization Phase: On Day 1, animals were topically sensitized with 10% DNFB distributed among the left and right ears, and left and right medial thighs. Challenge Phase: On Study Days 14, animals were challenged on designated dose sites with 1% DNFB. Dose administration: Approximately 2-4 hours post challenge, dose sites on the right side of the animal were treated with 1% hydrocortisone. Dose Site Scoring: Dose sites were scored for erythema and edema using three point grading scales (maximum total score: 6) during the course of three days post-challenge. Histopathology: Dose sites from one animal of each breed of minipig were processed to slides, stained with H&E, and evaluated by a pathologist for signs of dermatitis (24 hours post-challenge). Results: Mean summed irritation scores peaked at 24 hours in the Yucatan (Mean +/- SEM: 2.6 +/- 0.6), and at 48 hours post-challenge in the Hanford (Mean +/- SEM: 3.6 +/- 0.3) and in the Sinclair (Mean +/- SEM: 1.6 +/- 0.8). At 72 hours irritation was still
present, but moderate in nature (average: Hanford: 1.6; Sinclair: 1.0; Yucatan: 1.0). Hydrocortisone treatments did not affect the severity or the course of the allergic response. Histopathological findings were similar and consistent with lesions associated with contact dermatitis in all three animals, and included minimal to moderate epidermal necrosis and acanthasia, edema, eosinophilic and mononuclear infiltrates in the superficial and deep dermis. Conclusion: Moderate to prominent dermatitis was induced in Yucatan, Hanford, and Sinclair minipigs using 10% DNFB sensitization, followed by 1% DNFB challenge. Treatment of select dose sites with 1% hydrocortisone 2-4 hours post challenge with 1% DNFB did not reduce overall irritation scores in any breed of minipig. Length: less than 2,300 characters, all included but space.

2702 Definition of an In Silico Protocol for Skin Sensitization

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In silico assessments are an important component of toxicology. However, standardized protocols for the conduct and interpretation of in silico methods are lacking. A consortium comprising of over 55 diverse organizations has published a general strategy for developing in silico protocols to assess toxicological endpoints such as genetic toxicity, carcinogenicity, acute toxicity, reproductive toxicity, developmental toxicity, neurotoxicity, irritation/corrosion, etc. The purpose of the in silico standardization project is to generate, record, communicate, and archive results in a uniform, consistent, and reproducible manner, while fostering the use and reducing the burden on both industry and regulators to justify the use of these methods. Incorporating standardized principles into the use of in silico methods supports a more transparent analysis of the results and mitigates “black box” concerns. Here, we report on efforts to develop an in silico protocol for skin sensitization. Relevant effects/mechanisms are identified for the skin sensitization endpoint and a hazard assessment framework was established. Rules and principles are defined for integrating in silico predictions with in vitro data to derive a comprehensive assessment of skin sensitization. A) prediction of skin sensitivity, B) events in keratinocytes, dendritic cells, and rodent lymphocytes; and C) skin sensitization in vitro, rodents, and humans. In addition, a new software platform was developed to allow content searching against high-quality specialized databases and integrate models developed in compliance with the published protocols. Statistical models were developed for the Local Lymph Node Assay (balanced accuracy of 82.5%) and Direct Protein Reactivity Assay (balanced accuracy of 81%). Case studies illustrate the utility and flexibility of the hazard assessment framework. Standardization of in silico tool use and interpretation of results greatly reduces the burden on both industry and regulators by providing confidence in and justification for the use of these types of integrated approaches.

2703 Evaluation of OECD Accepted In Vitro Methods for Assessment of Skin Sensitizing Potential of Agrochemical Formulations

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Limited information exists on the predictivity of in vitro methods for the assessment of skin sensitizing potential of complex agrochemical formulations, despite the regulatory requirement for testing of these materials for classification and labelling purposes. The objective of this study was to explore the performance of OECD approved test methods in determining the skin sensitization potential of 7 agrochemical formulations with existing Local Lymph Node Assay (LLNA) and/or Buehler test data. Prior to testing the formulations, the proficiency of the laboratory to perform the Direct Peptide Reactivity Assay (DPRA), KeratinoSens®17C and Human-Cell Line Activation Test (h-CLAT) was confirmed successfully using the proficiency materials as outlined in the OECD Test Guidelines 442C, 442D and 442E, respectively. Of the formulations selected, four were known skin sensitizers (two emulsifiable concentrates [EC], one flowable concentrate for seed treatment [FS] and one suspension concentrate [SC]) and three were non-sensitizers (1 SC, 1 FS and 1 water dispersible granule [WDG]). A single average molecular weight (MW) for each formulation was calculated by considering the MWs of each component (including active/non-active ingredients and approximate MW for polymers), and their individual proportions resulting in MWs for testing ranging from 177 to 2260 kDa. Solvent selection and solubility assessments were performed for each formulation and KeratinoSens®17C and h-CLAT using the MTT assay and formulation doses adjusted to achieve >70% cell viability across much of the dose response curve. As such, starting doses of 1000μM to 2μM and 1.25 to 1.5-fold dilution steps, were applied depending on solubility and cytotoxicity of the formulation. Preliminary data suggest that the approach taken resulted in correct identification of 3/3 non-sensitizers, but only 1/4 sensitizers (formulation SC; IC50 > 0.4μM; IC50 > 2.0μM) due to cytotoxicity/solubility issues and that these issues are likely attributable to the agrochemical-co formulation components. Since keratinocyte activation represents only 1 key event of the skin sensitization Adverse Outcome Pathway, suitability of all 3 OECD accepted in vitro assays is being assessed.

2704 Applying In Silico Approach to Unravel Mechanism of Toxicity of Contact-Sensitizing Hair Dye Components

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Fifteen to 20% of the general population have allergic contact dermatitis as a clinical manifestation of contact sensitization, making this skin disease a major health problem. Screening for contact-sensitizing chemicals is highly relevant, particularly in dermatology. The use of animal tests for this purpose is constrained by ethical considerations, need for high-throughput screening and legislation (e.g., for cosmetics in the European Union). Although hair dye substances, that are strong or extreme sensitizers, are very common in consumer hair dye products on the European market, dermatitis patients are generally patch tested with one substance only, namely N-phenylenediamine. Therefore, it is not possible to accurately estimate the overall burden of hair dye allergy in dermatitis patients. The goal of the study was to develop knowledgebase and software tool that can be used to predict potentially harmful interactions between chemicals as an alternative to animal test methods. From the public domain and proprietary company datasets we collected data on 303 commercial hair dye formulations and their potential contact sensitizers such as health and beauty aids, household products, drugs and food. Our software generates networks of interacting chemicals, genes, proteins, pathways, and biological effects, providing extensive information on chemicals and their biological effects on organisms. In order to test our approach, we used the three ingredients most commonly used in permanent hair dye formulations (N-phenylenediamine, PPD; Resorcinol, RES and hydrogen peroxide, H2O2). We showed how simultaneous exposure may result in one chemical augmenting the toxic effects of the other. Our computational tool shows that RES and PPD target molecules that are involved in inflammation and allergy reactions like cytochrome P450 protein CYP1A1, CYP3A4, prostaglandin synthase PTGS2, chemokine receptors CXCR4, CCR6 and cytokines IL8, ILS. Further, our system identified correlation between these two chemicals and various skin conditions such as skin sensitization and skin inflammation. Bioinformatics analysis provides the molecular mechanism of how exposure to these hair dye components can induce skin sensitization, and putative explanation of additive and/or synergistic effect observed with these three chemicals.

2705 A Tier-Based Skin Sensitization Testing Strategy for Personal Care Products

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Tier-based testing strategies allow for a comprehensive evaluation of product safety in a cost and time efficient manner. Currently, there is a lack of formal testing requirements and standardized tier-based testing strategies for cosmetics and personal care products, especially for complex mixtures. The goal of this project is to develop a tier-based testing strategy for skin sensitization potential of personal care products. An on-market hair cleansing conditioner containing 23 ingredients was evaluated for skin sensitization potential using a proposed tier-based testing strategy for personal care products. The first tier of evaluation utilized the OECD QSAR Toolbox for an in silico evaluation of the skin sensitization potential of the identified ingredients with defined chemical structures. Subsequently, tier two testing utilized the OECD 442C in vitro guideline test (Direct Peptide Reactivity Assay [DPRA]) to evaluate identified ingredients with skin sensitization potential from tier one. To date, the OECD 442C in vitro guideline test has not been validated for food products. However, tier three testing adapted the OECD 442C in vitro guide- line to evaluate the skin sensitization potential of the total product mixture. The results from tier one in silico testing revealed four structural alerts for skin sensitization: methylchloroisothiazolinone, methylisothiazolinone, panthenol, and stearamidopropyl dimethylamine. Tier two in vitro testing of the individual structural alerts in tier one testing were not sensitizing at the concentrations tested. Tier three in vitro testing was consistent with individual ingredient evaluation; the formulated product was not
sensitizing. Together, the results indicated that although several ingredients were identified as potentially sensitizing, based on structural alerts from in silico testing and subsequent in vitro testing revealed that the individual ingredients and the product were non-sensitizers at the concentrations tested.

2706 An In Vitro Assay Platform to Assess Skin Sensitization Potential of Chemicals in Subset of Tox21 10k Library
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Skin sensitization, a toxicological endpoint, is known to be the initiation step of allergic contact dermatitis (ACD). Measurement of sensitization potential is a necessary step in the safety evaluation of both industrial and consumer products. In order to reduce and replace in vivo local lymph node assay (LLNA) gradually being replaced by in vitro cell-based assays. This study used several in vitro assays and 3D skin tissue equivalents to assess skin sensitization potential of topically used compounds. The Nrf2-ARE pathway has a well-established role in the detection of sensitizing chemicals with reactivity towards the cysteine residues in Keap1 protein. A total of 354 topically used compounds from the Tox21 10k library and 128 reference compounds were screened in an Nrf2-ARE-Luc reporter assay. Active compounds identified from Nrf2-ARE-Luc reporter screen were further tested for effects in the inflammation related assays that measure IL8 and IL1B expression in THP1 cells or primary keratinocytes. Finally, selected compounds were tested for concentration-response effects in activating dendritic cells by measuring the induction of cell surface expression of CD86 and CD54 by compounds on THP1 cells, as analyzed by flow cytometry. The ranking order of tested benchmark chemicals revealed that our test battery was able to distinguish strong from weak sensitizers. Some sensitizers tested in this study, such as 2,4-dinitrochlorobenzene (DNCB), methyl-dibromo glutaronitrile (MDBG) and Diphenylcyclopropenone (DPCP) are also contact irritants. We further compared the activity of these compounds in test battery assays with cytokine profiling assays in 3D bio-printed epidermis equivalents. The allergenic properties of the chemicals appeared to dominate over interactive properties since the effective concentration to induce sensitization was one magnitude lower than the irritation threshold. This high throughput test battery of assays provides a relevant tool to quickly detect sensitizers. The data generated from these in vitro assays provide a resource for predictive modeling and hazard identification.

2707 In Vitro Irritation Potential of Cyanotoxins in Reconstructed Human Skin
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Cyanobacteria are microorganisms that occur naturally in both fresh water and marine environments. They are found at higher densities in eutrophic waters. Cyanobacteria can rapidly increase under growth-enhancing conditions of sunlight, elevated temperature and increased loading of anthropogenic nutrients such as nitrogen and phosphorus, resulting in harmful algal blooms (HABs). HABs have been detected in all 50 states. Many cyanobacteria are capable of producing cyanotoxins. Microcystins and cylindrospermopsin are some of the classes of cyanotoxins formed, with the microcystins the most commonly detected. Human exposure to cyanotoxins can occur through drinking water, recreational activities (e.g., swimming, bathing), the aquatic food web, terrestrial plants, and food supplements. Human exposure to cyanotoxins are known to cause skin rashes, gastrointestinal, and nervous system, liver and kidney damage. The objective of this study is to assess the in vitro dermal irritation potential of microcystin LR and cylindrospermopsin. The in vitro Epiderm Skin Irritation Test (EPI-200-SIT MatTek, Ashland, MA., USA) is a 3D cell culture (referenced in the OECD Test Guideline 439 for skin irritation) that consists of organized basal, spinous and granular layers, and a multilayered stratum corneum containing intercellular lipid layers arranged in patterns analogous to those found in vivo. The tissue is derived from normal epidermal keratinocytes from neonatal foreskin. This tissue is also known as reconstructed human skin. Tissues (3/dose) were exposed to microcystin-LR (M-LR) at concentrations of 2, 20, 100, 200, 1000, and 2000 µg/L and cylindrospermopsin (CYN) at concentrations of 4, 40, 200, 400, 2000, and 4000 µg/L prepared in sterile water. The highest concentrations were 500-fold US EPA’s recommended recreational criteria or swimming advisory levels using a spectrophotometer. In this assay, at both the 1 and 24 hr timepoints, neither cyanotoxin was determined to be an irritant at any tested concentration. The conditions of this experiment, M-LR and CYN are not dermal irritants This abstract does not represent US EPA policy.

2708 Acute Dermal Irritation Response in White Sinclair and Hanford Minipigs
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Acute dermal irritation was induced and characterized in white Sinclair and Hanford minipigs using a variety of known human irritants at increasing concentrations (N = 2 per breed, 3-4 months old, all females). During acclimation the areas of the dorsum of the minipig to be tested were shaved, and the topical dermal challenge sites were marked. Animals were administered 0.5 mL of test compound on gauze to a 4 cm² area for 4 hours. Following dose administration, the gauze was removed (time 0) and animals were scored using a Modified Draize Scoring System for erythema (0-4) and edema (0-4), with a maximum score of 8. Scoring was conducted at 1, 24, 48, and 72 hours after test article removal. Materials tested were as follows: sodium hydroxide (0.5, 1, 2, and 4%), formaldehyde (0.8, 2, 4, and 8%), benzenzalkonium chloride (7.5, 15, 30, and 45%), and sodium dodecyl sulfate (20, 40, 60, and 80%). Results: The data indicate that all concentrations of formaldehyde and sodium dodecyl sulfate failed to show irritation in both minipig lineages. All concentrations of sodium hydroxide failed to show irritation in the Hanford minipigs, while 4% sodium hydroxide caused erythema (scores of 1 or 2) starting at 24 hours in both Sinclair minipigs. Benzenzalkonium chloride at 20% was shown to induce edema (scores of 1 or 2) in Hanford minipigs, however, benzenzalkonium chloride at 45% showed no difference between the lineages. Conclusion: When exposed to either sodium hydroxide or benzenzalkonium chloride, two known human irritants, white Sinclair minipigs display more irritation (based on erythema and/or edema scores) when compared to Hanford minipigs. At a certain irritation threshold (e.g. 45% benzenzalkonium chloride), there was no difference between the lineages. Although more testing needs to be performed to confirm the results, these early tests suggest that the white Sinclair minipig may be more sensitive to potential irritation and therefore a better animal model for human responses to chemicals. Length: less than 2,300 characters, all included but space.

2709 Validation Method to Detect Formazan Salt Using High-Performance Liquid Chromatography to Evaluate Irritation and Corrosion Assays in 3D Human Epidermal Model
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The disclosure of the 3Rs principle drove to the establishment of a large range of in vitro methods for different applications, especially for the safety evaluation of new chemical compounds. Considering that, OECD has published two widely used guidelines, OECD 431 (2016) and 439 (2015), for skin corrosion and irritation, respectively. For both tests, evaluation of the effect is based on the color produced by the generation of formazan salt, a well-known indicator of cell death. However, colored substances such as hair dyes are difficult to assess due to color interference. The aim of this study was to propose and validate a method for the determination of the salt of formazan in our “in house” Reconstruction Human Epidermis (RHE) model using HPLC according to the US FDA 2018. For that, we used a LC-10AT VP (Shimatzu) with UV-vis detector SPD-10A VP (Shimatzu) and the chromatographic conditions: mobile phase acetonitrile-water (80:20 v/v), Lichrospher 100® RP-18 (5µm) column; LichroCART® 125-4 HPLC-Cartridge, flow rate 1,2mL/min. Chromatograms were processed at a wavelength of 555 nm. For linearity, we prepared a standard solution of formazan in isopropanol with different points (0.5; 2; 20; 50; 100; 200 µg/mL). Precision and accuracy were performed using 3 different concentration and 3 independent calibration curves (intraday and interday assays), using 5 replicates. Solutions of each concentration were dried and resuspended in mobile phase prior to injection. In order to evaluate the matrix effect, we used 5 RHE fortified with 3 concentrations of formazan (2; 20 and 100 µg/mL), followed by the recovery determination. For selectivity, using RHE, an extraction step was necessary to evaluate interference from matrix. As results, we observed a linearity with r =0,99; recovery up to 85%; accuracy and precision with standard deviation <15%. Additionally, the method is validated and suitable for analysis of corrosion and irritation in RHE after exposure to colored compounds. Financial Support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).
Establishment of Skin Irritation and Decontamination of TMAH Splashes: Genotoxic Effects Induced by Hair Dyes in Development of a Method to Measure Systemic Toxicity. Confirmation with pH with hypertonic, amphoteric solution could limit tissue diffusion and thus of the corrosive base effect of TMAH and of decontamination for returning pH to be ~10 times greater: 140, 250, or 1,100 ml. These studies highlight the role to 2.5, 5, and 25% TMAH, respectively. With tap water, added volumes needed it was necessary to add, 8, 15, and 100 ml of hypertonic, amphoteric solution appears after 600 ml are added. To achieve a physiologically tolerable pH of ~9, decontamination, a precipitate occurs after 18 ml are added and then disapperition, a precipitate occurs and persists. With hypertonic, amphoteric solution permeable membrane more rapidly than the 2.5 and 5% concentrations: 2 vs. either tap water or an hypertonic, amphoteric solution using a dosing tech deposited on the top of the membrane in contact with a 6 ml compartment of diffusion of TMAH concentrations of 2.5, 5, and 25% (Acros; pH =14; pH =13.6) through the skin. 3 drops of the contaminant were deposited on the top of the membrane in contact with a 6 ml compartment of sodium chloride. Simulation of TMAH decontamination was performed using either a single in vitro test or in vitro test can replace accepted animal based results. Significant efforts have been made to employ RhE models as a standalone skin sensitization assay. For example, genmic arrays (eg. SENS-IS) provide powerful tools for key event (KE) genomic based biomarker assessment. Here, we present a proteomic array using electrophoresimecence multiplexing technology as a single assay to assess the regulation of key biomarkers associated with known KE for skin sensitization. Data from concurrent detection and quantification of KE biomarkers is presented as a proof of concept multiplex approach to the assessment of skin sensitization.

Decontamination of TMAH Splashes: In Vitro Experiments

L. Mathieu1, K. Padois1, H. Couzdelou1, F. Rondinelli1, J. Blomet1, and A. H. Hall2,1. 1Laboratoire Prevoir, Valmondois, France; 2Toxicology Consulting and Medical Translating Services, Azle, TX; and 1University of Colorado Denver, Denver, CO.

Tetramethylammonium hydroxide (TMAH) is a quaternary ammonium compound and also a strong corrosive base. TMAH is widely used in the semicon- ductor and photovoltaic industries as an etchant and developer. It dissociates into the TMA+ and OH- ions, the latter causing chemical skin/eye injuries and the former felt responsible for systemic toxicity including reported fatalities. It is plausible that dermal injury from the OH- ion may increase tissue penetration of the systemically toxic TMA+ ion. In vitro experiments were performed using a semipermeable cellophane membrane (30 g/ml, 20.8 μm) to mimic diffusion of TMAH concentrations of 2.5, 5, and 25% (Acros; pH =15.6; pH =14; pH =13.6) through the skin. 3 drops of the contaminant were deposited on the top of the membrane in contact with a 6 ml compartment of sodium chloride. Simulation of TMAH decontamination was performed using either a single in vitro test or in vitro test can replace accepted animal based results. Significant efforts have been made to employ RhE models as a standalone skin sensitization assay. For example, genmic arrays (eg. SENS-IS) provide powerful tools for key event (KE) genomic based biomarker assessment. Here, we present a proteomic array using electrophoresimecence multiplexing technology as a single assay to assess the regulation of key biomarkers associated with known KE for skin sensitization. Data from concurrent detection and quantification of KE biomarkers is presented as a proof of concept multiplex approach to the assessment of skin sensitization.

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There are a number of approaches to evaluate the safety of a personal care or cosmetic product, including clinical testing. Recently, there have been consumer reports of scalp dermatitis following the use of a commercially available cleansing conditioner. The aim of this study was to evaluate the skin tolerability of 6 commercially available hair cleansing products. A double-blind, randomized, controlled, IRB-approved clinical trial of 6 hair care products (HCPs) using Repeat Open Application Tests (ROATs) and Semi-Open (SO) patch tests was performed. For the ROAT, 150 healthy adult volunteers applied 0.2 ml of product to 6 separate, randomized locations on the forearms per standardized protocol. Exposure time was increased (5-15 minutes) over 5 weeks. Participants and investigators were blinded to product locations. Application was discontinued if a ROAT score $\geq 6$ (10 maximum). SO testing was performed at week 4 and graded at week 5 in a double-blind randomized fashion. Primary outcomes included ROAT and SO scores for each HCP. Of the 150 individuals that have completed the study, 84.7% are female, with a mean age of 48.4 years. 41.3% of the study participants had a history of either atopic dermatitis, asthma, or hay fever. Preliminary analysis (performed with study blind intact) found that 5.3% of participants achieved stopping point (total ROAT$\geq6$) for one HCP. That same product resulted in ROAT scores of $\geq 1$ and SO score $\geq 4$ for 42.7% and 3.1% of participants, respectively. In contrast the remaining 5 HCPs had $\leq 3.3\%$ with ROAT scores $\geq 1$ and $\leq 3.6\%$ with SO score $\geq 4$. There is a significant difference in tolerability of the 6 HCPs evaluated as measured by ROAT and SO.

TNIP1 protein is a widely expressed, cytoplasmic inhibitor of inflammatory signaling initiated by membrane receptors for pathogen-associated and damage-associated molecular patterns (PAMPs and DAMPs) coming from endogenous and/or environmental sources. TNIP1 deficiency occurs in inflammatory skin diseases such as lupus and psoriasis, possibly sensitizing cells to such stimuli. Recently, we published TNIP1 deficiency in keratinocytes sensitizes them to experimentally-defined PAMPs and DAMPs thus promoting hyper-responsive expression and secretion of inflammatory markers (e.g. IL-8, IL-6 and TNFα). Thus, TNIP1 deficiency with subsequent stimulation establishes intrinsic (gene expression) and extrinsic (cytokine milieu) changes which we predict may modulate global tissue events such as wound healing. In this study, we examined the wound healing parameters of cell survival, migration, and expression of other inflammation-related genes. Specifically, we used our cell culture model of TNIP1 deficient keratinocytes to investigate stimulating toll-like receptor 3 with the agonist poly IC, a representative dsRNA PAMP/DAMP. Our studies revealed increased expression of antimicrobial (e.g. $\alpha$100a family) and wound healing (e.g. IL-8, CCN2) associated genes, suggesting potential benefit of increased inflammatory response from TNIP1 deficiency. Unexpectedly, poly IC challenge of TNIP1 deficient cells restricted re-epithelialization and induced cell death as compared to TNIP1 deficiency alone. Intriguingly, we detected not only increased expression for genes associated with cell death and inflammasome activation (e.g. caspase 1, IL-1β) but strikingly also for A20, a protein that represses cell-death signaling downstream of membrane receptors. Despite this compensatory increase in A20 mRNA, we found evidence of a decrease in its protein activity. Thus, an underlying mechanism for the hyper-responsive phenotype of TNIP1 deficient keratinocytes following DAMP/PAMP stimulation involves not only an increase in inflammatory gene expression but also likely includes deficiencies in adequate compensatory responses such A20 activity.
Humans are exposed to ultraviolet radiation (UVR) throughout their lives, which has both beneficial and detrimental effects. UVR is essential for vitamin D synthesis, but high exposure can cause painful sunburns and increase one’s risk of skin cancer due to its high mutagenicity. Sunscreens have been developed to combat UVR’s negative effects, and are even more highly encouraged for at risk populations. While sunscreens have been shown to be effective at reducing the risk of skin cancer, there is evidence to suggest that some UV filters may have hormonal and endogenous compounds, as well as further toxicological studies on these chemicals essential. We hypothesized, based on structural aptitude, that some UVR filters found in sunscreens may be able to modulate Aryl hydrocarbon receptor (AhR) signaling. The AhR is a ligand-activated transcription factor that plays a significant role in the metabolism of various endogenous and exogenous compounds, as well as regulating the immune system. Interestingly, the endogenous AhR agonist 6-formylindolo-3,2b-carbazole (FICZ) is thought to be produced in the skin after UVR exposure. To test our hypothesis, we utilized the H1L1.1c2 mouse hepatoma AhR reporter cell line to screen UVR filters for activity as an AhR agonist or antagonist. While the UVR filters that we have tested thus far show no agonist or antagonist activity towards the AhR, co-treatments of the UVR filter octinoxate and FICZ resulted in a potentiation of AhR activation that was greater than what FICZ alone was able to elicit. We repeated these treatments on an immortalized keratinocyte cell line (HaCaT) and analyzed RNA expression of cytochrome P4501A1 (CYP1A1), a known AhR target gene, via real time PCR (qPCR). Our results showed that this potentiation also occurs in keratinocytes. Based on our results, we hypothesized that octinoxate acts as a CYP1A1 inhibitor, resulting in a reduction in the rate of FICZ metabolism and sustained activation of the AhR. To test this hypothesis, we performed an Ethoxyresorufin-O-deethylase (EROD) assay as well as a biochemical CYP1A1 inhibition assay (Promega). The results of both assays strongly suggest that octinoxate is able to inhibit CYP1A1 activity. While there is more to be done, our research has produced significant evidence to suggest that octinoxate has off-target effects on normal AhR signaling via CYP1A1 inhibition, which has many potential implications for skin function.

2719 Beauty Sleep: Skin Collagens Regulate Sleep in Response to Cell Stress in Caenorhabditis elegans


When animals are sick or injured from an exposure that results in cell stress, they respond by sleeping; such behavior is called sickness or stress induced sleep (SIS). The pathway regulating SIS may be conserved from nematodes to vertebrates. Cellular stress in C. elegans leads to activation of the epidermal growth factor (EGF) receptor on the ALA neuron, which releases neuropeptides. ALA neuropeptides induce SIS behavior, which consists of immobility and feeding quiescence, elevated arousal threshold, and rapid reversibility. While much is known about ALA activation and its downstream mechanisms, the SIS pathway upstream of EGF remains poorly understood. This part of the pathway is the focus of our work. We found that mutants lacking the cuticular collagens DPY-5, DPY-10, or DPY-13 show impaired feeding and movement quiescence following exposures to ultraviolet (UV) irradiation or heat shock. Feeding was measured by counting the number of pharyngeal pumps in 10 seconds. Movement quiescence was measured using machine vision algorithms. The loss of skin collagen genes results in short, fat worms, a morphological phenotype referred to as Dumpy or Dpy. To determine where the dpy mutation acts in the SIS pathway, we crossed each of them into an inducible-EGF background. Each of the three dpy mutants showed normal or enhanced SIS following the induction of EGF, suggesting that these genes act upstream of EGF. To determine if mutants with a Dpy phenotype due to mutations other than collagen genes are important for SIS, we tested mutants in dpy-19, which encodes a C-mannosyltransferase. dpy-19 mutants had normal SIS following both UV and heat shock, suggesting that specifically collagen gene disruption and not the Dpy phenotype explains the impairment in SIS in the dpy-5, -10, and -13 mutants. To determine whether disruption of the cuticle in the absence of a Dpy morphology affect SIS, we tested mutants in bus-8, which encodes a glycosyltransferase that is important for cuticle integrity. bus-8 mutants do not have a Dpy phenotype. We found that bus-8 mutants are not deficient in SIS, suggesting that disrupting the cuticle is not sufficient to impair SIS, but that collagen disruption specifically is important. We conclude that cuticular collagen disruption impairs stress induced sleep. This information informs our understanding of the relationship between skin collagens and fatigue/sleepiness associated with sickness in humans.

2720 Oral Ingestion of a Novel Oxygenating Compound, Ox66, is Non-toxic and Results in Increased Oxidation

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Ox66TM is a novel solid-state oxygenating compound. In order to support the use of Ox66TM as a potential oxygenating supplement to alleviate conditions caused or exacerbated by hypoxia, this study evaluated the safety of Ox66TM, its ability to withstand the conditions of the digestive tract, and its potential to increase oxygenation in the mesentery in rats. The toxicity of Ox66TM was evaluated by performing acute (10-day) and chronic (90-day) feeding studies on rats. The stability of the compound in the digestive tract was evaluated via ex vivo simulated digestion and subsequent CFDA viability assay on gut epithelial cells. Its capacity for oxygenation in the mesenteric microcirculation was determined by interstitial fluid pressure (P,interstitial) and oxygen measurements upon injection into the small intestine of rats. No toxicity was found associated with acute or chronic oral administration of the compound in rats, and the compound was able to withstand the environment of the digestive tract in vivo. Based on the animal feeding study, the NOAEL was considered to be 1000 mg/kg/day. This proof-of-concept study further demonstrates the potential of Ox66TM to function as an oxygenating supplement that might be useful for treating either pathological hypoxic-related conditions or to improve oxygenation levels during or after exercise under healthy conditions.

2721 Application of New Approach Methodologies (NAMs) to Address Data Gaps in Human Risk Assessment: 2-Amino-2-Methylpropanol (AMP) Case Study

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2-amino-2-methylpropanol (AMP™) is an organic chemical widely used as a dispersant and neutralizer in the paints and coatings, and personal care industry. In previous evaluations, high oral doses of AMP in rat and dog repeat-dose studies induced liver steatosis and an early post-implantation loss in an OECD 422 developmental screening study in rats. As a result, several regulatory agencies have requested additional animal studies to better characterize the risk to human health. The current work utilized New Approach Methodologies (NAMs), in silico and in vitro approaches, to address uncertainties in the human risk assessment. To leverage existing human data on a widely used over-the-counter (OTC) diuretic drug Pamabrom, the ability to read-across was substantiated by conducting a bioequivalence study with AMP and Pamabrom. This, in tandem with in vitro methods combined with in vivo to in vivo extrapolation (IVIVE) to estimate an in vivo human point of departure, was conducted to strengthen the human risk assessment and address the data gaps raised by the regulatory bodies. The human data from the Pamabrom and the in vitro/IVIVE work addressed the uncertainty in the risk assessment process, with respect to extrapolation of results from animal studies to humans, as well as the differences in sensitivity to effects on target tissues between species. This additional data strengthened the weight of evidence regarding the overall safety of the chemical having a long history of safe use, thus preventing additional animal testing for hazard assessment of chemicals.

2722 Next Generation Products Induce Lower Biological Activity Than Combined Cigarettes: A Comparison of Aerosol Chemistry and In Vitro Toxicity

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Tobacco smoking has long been recognised as a major cause of disease. While public health bodies recommend complete cessation as the best action smokers can take to improve their health, some organisations begin to recognise the harm reduction potential of alternative products to smokers who are unwilling or unable to quit. There are many next generation products (NGPs) commercially available aiming to provide an alternative to smoking, with a significant reduction in harm. The study objective was to compare the chemical composition and in vitro toxicological activity of different NGP aerosols with conventional cigarette smoke. Products investigated were the Kentucky reference cigarette (3RAF), a tobacco heated product (THP), a hybrid product (THY) and a myblu™ vapour product (1.6% [w/w] nicotine; tobacco flavour). The smoke/aerosols were generated using the Health Canada Intense smoking regime for 3RAF and THP (55mL/2s/30s) and the CORESTA Recommended
Method No. 81 (55mL/3s/30s; square wave puff profile) for HYB and mybelle\textsuperscript{a}. The collected whole smoke and aerosols were tested under the CORESTA regulatory in vitro toxicity assays: neutral red uptake (BEAS-2B) for cytotoxicity, Ames (TA98, TA100) for mutagenicity and in vitro micronucleus assay (V79) for genotoxicity. Chemical analysis of all the NGP aerosols tested revealed substantial reductions in aerosol constituents when compared with conventional cigarette smoke. For example, the WMO 9 priority toxicants were below the level of detection in mybelle\textsuperscript{a} aerosol corresponding to a >99% reduction compared to 3RAF. All NGP aerosols demonstrated marked cytotoxicity reductions compared to cigarette smoke on a per puff basis. 3RAF smoke was highly mutagenic in the Ames test and induced significant genotoxicity after 1 puff. This cross-section represented a significant response in TA98 only, and induced genotoxicity after 24 puffs. In contrast, neither HYB nor mybelle\textsuperscript{a} aerosols were mutagenic or genotoxic (up to 100 puffs) under the test conditions. The data shows clear differences between 3RAF cigarette and NGP emissions and in vitro toxicity. NGPs should be considered to have the potential to reduce smoking-related disease risks.

### 2723 Use of Alternative Test Methods to Assess the Sensitization Potential of Commonly Used Botanical Extracts Found in Cosmetic Products

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Botanical ingredients are increasingly used in cosmetic products. Because of increasing limits internationally on animal testing, the sensitization potential of such ingredients will depend more heavily on alternative test methods. We have undertaken a study to evaluate the skin sensitization potential of a number of botanical extracts and ingredients from 	extit{Matricaria chamomilla} (German chamomile), a commonly used botanical in skincare products, using \textit{in chemico}, \textit{in silico}, and \textit{in vitro} methods that have previously been developed and/or validated for decision-making. Initially, (quantitative) structure-activity relationship (Q)SAR models built on human data as well as murine local lymph node assay (LLNA) data were applied to screen 246 chemical constituents of German chamomile for their skin sensitization potential, and the results were combined in a weight-of-evidence (WoE) predictions. Based on these (Q)SARs (\textit{in silico}) WoE prediction results, 30 compounds were chosen for further \textit{in chemico} and \textit{in vitro} testing using the Direct Peptide Reactivity Assay (DPRA), KeratinoSens\textsuperscript{TM}, the human Cell Line Activation Test (h-CLAT), as well as a recently developed chemical high-throughput assay using dapsyl cysteamine (HTS-DCYA). Among the compounds tested to date, 2,5-dihydroxybenzoic acid, quercetin, epicatechin, luteolin and caffeic acid were found to be reactive both in the DPRA and HTS-DCYA, which are both designed to simulate the molecular initiating event in the skin sensitization adverse outcome pathway. The phenolic constituents appear to be more reactive than the terpenoids constituents of chamomile. On the other hand, the lipophilic compounds such as guaiazulene, herniarin and bisabolol, were not chemically reactive but they did elicit an increase in the expression of at least one of the two protein markers on the cell surface in the hCLAT assay. Future testing of these assays including KeratinoSens\textsuperscript{TM} will be performed to investigate the ability of German chamomile constituents to activate inflammatory responses mediated by the Kep-ARE-Nrf2 pathway in keratinocytes.

### 2724 Improving Safety Assessment Weight-of-Evidence with Poison Center Data

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Safety assessments rely on weight-of-evidence (WoE) analyses to integrate human effects and toxicology information. Human effects data are often limited. Poison Centers (PCs) respond to millions of calls each year, including accidental and intentional misuse of various consumer products. Such data might support evaluation of exposure concentrations, dose-response, and severity of health effects. PC records were evaluated for alcohol-based hand sanitizer (ABHS). Details from 2521 PC calls regarding exposure to ABHS were evaluated for information on the route of exposure, estimation of the amount consumed, presence and severity of health effects, patient characteristics (e.g., age, weight), product characteristics (e.g., brand, color, scent), packaging characteristics (e.g., dispenser type, package size), and formulation characteristics (e.g., active ingredient type, concentration). Retrospective evaluation of the case narratives allowed for descriptive and trend analyses, but many of the (Q)SAR predictions could not be verified due to gaps in data collection during the initial PC consult and a lack of consistent follow-up to confirm patient outcomes. Reported clinical effects and severities suggest that accidental ingestions are inconsequential and do not result in serious health complications. Accidental ingestions of ABHS demonstrated a similar patient outcome profile to traditional hand soaps, with a lower rate of clinical effects when compared to ethanol-containing mouthwashes. These findings add to the WoE from studies of other ethanol-based consumer products of the retrospective analysis identified characteristics or scenarios that are more likely to lead to accidental or intentional misuse. 82% of ABHS cases were children ≤ 5 years of age and most (74%) ingested only a small amount (0-4 ml), defined as a “taste”. Children were 4.5 times more likely to consume more than a “taste” if they had direct access to the ABHS. However, much of the retrospective information is limited for use in quantitative dose-response analysis. Design and implementation of a prospective analysis would fill identified data gaps and improve the reliability of data capture for safety assessment through standardization of questions asked during PC calls and attempted follow-up on all reported incidents.

### 2725 Evaluating the Phototoxic Potential of a Hair Cleansing Conditioner

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Phototoxic dermal reactions may be produced by topical administration of exogenous chemicals. Chemicals that cause phototoxicity absorb ultraviolet (UV) light and transfer this energy to other molecules or form reactive free radicals that result in adverse reactions such as redness and blistering. No standardized test for phototoxicity is required under current US FDA regulations for personal care and cosmetic products; however, if UV exposure is anticipated following product use, evaluation for phototoxic potential may be necessary. The goal of this study was to utilize a validated phototoxicity test to evaluate the phototoxic potential of three on-market hair cleansing conditions to demonstrate this assay’s applicability to personal care and cosmetic products. The OECD 432 in vitro guideline study was utilized to evaluate the phototoxic potential of the hair cleansing conditioners. The hair cleansing conditioners were evaluated by a UV-visible light (VIS) spectral scan to ensure no significant absorption between 280 and 400 nm. Subsequently, Balb/c 3T3 mouse fibroblast cells were exposed to the hair cleansing conditioners and controls for one hour. Following the incubation, one set of treated 3T3 cells were irradiated with 4 minutes of 5 J/cm\textsuperscript{2} Solar Simulated Light (SSL), containing wavelengths of UVA and visible light with more than 99% of UVB blocked out. A duplicate set of treated 3T3 cells were kept in the dark. After UV irradiation, 3T3 cells were washed and incubated for 24 hours and cell viability was determined by neutral red uptake. The difference in cell viability between the SSL exposed and non-exposed 3T3 cells were used to determine the phototoxicity of the hair cleansing conditioners. Under the conditions tested, the hair cleansing conditioners were not phototoxic in the 3T3 neutral red uptake phototoxicity test, while the positive control was significantly phototoxic. Taken together, these results demonstrate that the tested hair cleansing conditioners did not have phototoxic potential.

### 2726 An Inter-species Comparison of the Triclopyr In Vitro and In Vivo Toxicokinetic Properties, for Risk Assessment Purposes

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Triclopyr is a synthetic auxin, a broad-weed herbicide, used in pasture and rice. Hence, external human exposure is possible via dietary and non-dietary route. Herein, we present a multi-faceted approach, utilizing newly generated data from \textit{in vitro} systems and existing internal data from \textit{in vivo} studies. This project includes academic and volunteer research volunteers, as well as \textit{in silico} predictions of systemic exposure to recapitulate oral gavage and dietary exposure profiles in various species, including humans. The oral absorption of triclopyr was complete in mammals as well as in human volunteers and, \textit{in vitro}, in a CaCo-2 cells experiment. \textit{In vitro} comparative metabolite usage under dietary and \textit{in vivo} confirmed lack of metabolism in humans, similar to other species. Excretion mechanisms, previously investigated in \textit{in vivo} renal clearance study in dog, are unique to that species, while a more rapid renal elimination in rat and human would correlate with known lower systemic levels compared to dog. Comparative \textit{in vitro} plasma protein binding confirmed that the plasma free fraction available for glomerular filtration is

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higher in dogs compared to rat and humans. Renal clearance was additionally investigated in primary proximal tubule cells to determine direction, magnitude, and transporters involved in mammals’ tubular excretion/resorption fluxes. Overall the results of these studies will be utilized to confirm that the triclopyr toxicokinetic profile of rats is more similar to humans, while dogs present a very species-specific excretion system of organic acids. These data are key to interpret toxicity features in rats. In addition, since the point of departure for triclopyr risk assessment are based on rat studies, the similarities between these two species greatly reduce uncertainties (and, possibly, safety factors) for the use in human health risk assessment.

**2727 Safety Testing of Bacteria: Challenges and Approaches to Translocation Testing in Studies Conducted in a High-Throughput Vivarium**

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Safety assessments for live microbial products, such as bio pesticides and probiotics, present unique experimental challenges for an in vivo testing laboratory. Among these is the assessment of potential for translocation - the migration of an organism from the gut to other parts of the body. For assessment of translocation endpoints during probiotic safety evaluation in 90-day rat gavage studies, blood samples and selected tissues are collected in the vivarium setting at necropsy, transported to a laboratory with biosafety cabinets, homogenized, and the homogenate plated and analyzed using appropriate conditions. Colonies are counted, scored by 8 morphological criteria, and sub cultured for subsequent molecular identification. A critical variable for analysis of translocation from the gut to the tissues is the potential for environmental contamination during tissue harvest. Even with the strict sanitization protocols of a modern, high-throughput vivarium, absolute sterility can be difficult to achieve. This is especially true when large numbers of animals are processed quickly to collect the many samples required for a thorough safety evaluation. During three recent 90-day rat gavage studies with probiotic test substances, techniques designed to minimize environmental microbial contamination were implemented but not evaluated by limit implemented. Enhanced vivarium practices included aseptic blood collection techniques, whole-animal and tissue sanitation procedures, and prioritization of tissue collection for translocation assessment over tissues intended for other endpoints. In addition to enhanced vivarium practices, it was determined for solid tissues that briefly submerging the samples in a sodium hypochlorite solution prior to homogenization provides effective surface decontamination without affecting identification and quantification of microbes within the tissue. In sections of chicken liver with application of a bacterial indicator strain, hypochlorite pretreatment reduced surface contamination by greater than one log in the absence of a statistically significant reduction in detectable bacteria injected into the liver. Implementation of these practices facilitates accurate and reliable evaluation of microbial translocation in large, multi-endpoint safety studies.

**2728 Effect of Percent S9 Fraction on Bacterial Background Lawn Assessment in Ames Assay Using 35mm Plate Spread Technique**

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The Ames assay is commonly used to evaluate the mutagenic potential of pharmaceutical and tobacco products for regulatory purposes. A significant increase in the number of His+ revertant colonies observed in agar containing Salmonella bacteria and a test chemical indicates mutagenicity. To assess cytotoxicity in this assay, thinning of the background bacterial lawn is microscopically determined. With the development of new in vitro exposure systems that permit continuous cell exposure to aerosols or smoke, the Ames assay method required modification by spreading the bacterial solution on top of agar in a 35 mm plate. Using this technique, we observed reproducible thinning of the bacterial lawn by limiting the amount of histidine/biotin (nutrients) in the absence of the S9 fraction. However, in the presence of 10% S9, background lawn thinning could not be seen. We hypothesized that a reduction in the amount of S9 in the spread solution on the agar plate would allow the microscopic assessment of lawn thinning while providing adequate metabolic activation. Therefore, the objective of this work was to determine if microscopic analysis can detect thinning of the bacterial background lawn on top of the agar with 5% S9 fraction. The Ames assay was performed using a 35 mm agar plate spread technique with a bacterial solution in the absence or presence of 5 or 10% S9. Salmonella strains (TA98, TA100, TA102, TA1535, TA1537 and TA97a) were used to determine the number of His+ revertants and lawn thinning after 48 hours of incubation. The effect of 5 or 10% S9 fraction on the positive and negative control values for revertant colonies were also determined. We observed the reproducible thinning of the background lawn by limiting the amount of histidine/biotin in the absence and presence of 5% S9 but not with 10% S9 fractions. Using 5% S9, the positive and negative control values for revertant colonies were within historical control ranges (similar to 10% S9) for all strains. We conclude that thinning of the background bacterial lawn was clearly observed in all strains using the Ames assay with spread techniques and presence or absence of 5% S9 and microscopic analysis. The use of 5% S9 fraction with the 35 mm plate spread technique should permit the assessment of both mutagenicity and cytotoxicity in vitro aerosol or smoke exposure.

**2729 Oral Toxicity Testing of the Novel Green Insensitive Munitions Explosives, 2,4-Dinitropropyrazole (DNP) and 2,4,6-Trinitro-3-bromoanisole (TNBA)**


The US Department of Defense (DoD) utilizes Composition B, which includes 2,4,6-trinitrotoluene (TNT), in artillery and mortar rounds. TNT has known occupational toxicity concerns and is a possible human carcinogen. Exposure to TNT can exhibit a variety of toxicities to the liver, blood, immune system, and reproductive system. Additionally Composition B does not meet current insensitive munitions (IM) requirements mandated by the DoD. As such, the focus of the Green Insensitive Munitions Explosive (GriMex) program is to develop an alternative IM formulation containing novel replacements for TNT that present fewer concerns to occupational exposures of TNT. 2,4-dinitropyrazole (DNP) and 2,4,6-trinitro-3-bromoanisole (TNBA) have progressed through this program as potential replacements for TNT. Dose verification and stability measurements determined that both compounds are stable in corn oil at room temperature for at least 5 weeks. The toxicity of these two compounds was tested via acute and 14-day subacute oral gavage dosing in Sprague-Dawley rats using corn oil as the vehicle. Acute dosing via the Stagewise Adaptive Dose Method resulted in LD50 values 444 (95% CI: 364-542) mg/kg and >2000 mg/kg for DNP and TNBA, respectively. Subacute dosing at 5 different dose levels for each compound produced a variety of compound-related toxicities. The results of this study will provide vital information in the effort to identify suitable replacements for TNT.

**2730 The US FDA Animal Rule and Standard for Exchange of Nonclinical Data (SEND)**


The Standard for Exchange of Nonclinical Data (SEND) is the newly required format for submissions of nonclinical data to the US Food and Drug Administration (US FDA) for carcinogenicity studies, and single and repeat dose toxicity studies. The framework of SEND was modeled off the older Standard Data Tabulation Model (SDTM) used for clinical submissions; both were created by the Clinical Data Interchange Standards Consortium (CDISC) with the goal of standardizing and digitizing data for the US FDA reviewer. SEND is not currently required for Animal Rule studies, however there is an increasing desire to use the SEND format for data visualization and warehousing purposes. Under the current SEND modeling paradigm, animal rule studies introduce several complications during the formulation and review of the dataset. For example, SEND has no ability to include Day 0, which is counter to the traditional design of many animal rule studies that use Day 0 for challenge doses (i.e., day of radiation, day of infection, etc). Additionally, modeling the challenge agent and test article within the Trial Domains are not demonstrated within the current guidelines, making best practices and standardization across different studies and sites unachievable. Since the current 3.0 guidelines do not allow the introduction of non-defined domains, information regarding challenge agent dosing, medical history, medical countermeasures, and microbiology samples will have to be included in existing domains, which will stretch their original purpose and incorporate non-controlled terminology. A CDISC Animal Rule team is currently working on drafting a guidance standard for Animal Rule studies. This guidance standard will be subject to US FDA and public input prior to being issued as a requirement.
Chemical Alternatives Assessment of Selected Phthalate Substitute Plasticizers: Carboxylates, Adipates, Citrates, and Trimellitates

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Phthalate esters are primarily used as plasticizers for flexible polyvinyl chloride (PVC) which, in turn, is used in many consumer products. Certain members of this chemical class, such as di-(2-ethylhexyl)-phthalate (DEHP), have been shown to cause reproductive and developmental toxicity. Because of their health concerns, six phthalate esters are now restricted from use in children’s products. As a result, a number of substances have been identified as alternative PVC phthalate plasticizers such as carboxylates, adipates, citrates and trimellitates. Although many of these alternatives show promising application potential, data regarding their potential toxicity are discordant and/or incomplete, and with limited studies conducted to characterize their environmental fate and toxicity. Towards this, we evaluated the human health and environmental hazards of nine commonly used non-phthalate ester plasticizers from various chemical classes. Their toxicity hazard profiles were compared to DEHP and a lower molecular weight phthalate, dimethyl phthalate (DMP). We utilized the GreenScreen® for Safer Chemicals (GS) tool to assess and compare hazard profiles of these plasticizers and identify critical data gaps. The GS assesses chemicals for 18 human health and environmental hazard endpoints to assign an overall benchmark (BM) score ranging from 1 (High concern) to 4 (Safe). A GreenScreen summarizes endpoint scores in a hazard summary table to allow for ease of visualization. Based on our investigation, two plasticizers were demonstrated to be good alternative to DEHP as they have the best GS BM scores (i.e., minimum BM score except for a data gap). These substances are bis(2-ethylhexyl) terephthalate (DEHT), the isomer of DEHP, and epoxidized soybean oil (EBSO), a vegetable oil-based plasticizer. Tributyl O-acetylcitrate (ATBC), a citrate-based plasticizer, also demonstrates low toxicity for human health endpoints. However, it poses a hazard for aquatic toxicity. The remaining investigated plasticizers including DMP all have a GS BM score of 2 due to their potential human health and environmental toxicity. To this end, this poster will outline the GS methodology and present in detail the results of the GS assessments for the assessed plasticizers including their chemical identities. This visual communication of hazard information for phthalate ester plasticizers will help formulators select less hazardous plasticizers and recognize important data gaps in each alternate chemical's dataset.
JP-8, a petroleum-based jet fuel, is currently the primary fuel used in US Air Force aircraft. Because the US Government is interested in lowering dependence on crude oil for military use, alternative fuels have been developed that would be either combined with or used in place of JP-8 for military operations. This study investigated the sensory irritation potential of the inhalation of aerosols and vapors from Amyris C15, an alternative fuel that is produced by a direct sugar to hydrocarbon process and is mostly the alkane farnesane. Sensory irritation is the most common harmful effect of inhaled airborne chemicals, and irritancy can be quantitatively measured in mice by reflex inhibition of respiratory rate. The concentration of a chemical that induces a 50% respiratory depression is termed the LD₅₀, and describes the sensory irritancy potential of that chemical. The testing method that was used followed the American Society for Testing and Materials guideline: Standard Test Method for Estimating Sensory Irritancy of Airborne Chemicals. Briefly, separate cohorts of Swiss-Webster mice were exposed nose-only to five concentrations of an Amyris aerosol/vapor mixture for 30 minutes. Respiratory rates were measured with a body plethysmograph during the exposure and 10 minutes post-exposure and compared to a pre-exposure baseline. Amyris exposure did not induce a respiratory rate depression at least 50% at any of the concentrations tested.

Several recent reviews and risk assessments have addressed the potential for cosmetic talc to cause disease, particularly ovarian and lung cancer and mesothelioma. These studies have generally focused on epidemiology studies (including studies of industrial talc-exposed populations) that have evaluated whether talc or its potential contaminants might cause these diseases. The issue of what (if any) contaminants might be found in cosmetic talc has been the subject of intense study for decades. There has not been a recent critical integrated effort to determine whether animal studies support a potential disease link between talc and/or its contaminants and cancer. We evaluated 17 animal studies of talc, identified through a combination of literature searches and compilation of previous reviews. In addition, we synthesized data from other animal studies that evaluated whether characteristics (e.g., fibrous form, length, dimensions, etc.) of some talc particles or contaminants might be a factor too in the cancer risk. Our critical review of talc-specific animal studies did not support that exposure to cosmetic talc products would result in an increased risk of cancer. Analysis of animal studies that evaluated whether potential contaminants of cosmetic talc such as fibrous talc or other non-asbestiform elongate mineral fibers (regardless of dimensions) also did not support an increased risk of cancer. The animal studies support the premise that high doses of cosmetic talc and/or its contaminants could be associated with non-cancer respiratory effects, such as inflammation and fibrosis. These observed effects are consistent with the type of health effects observed in animal studies of other nuisance dusts, and human reports that have found evidence of talcosis in cosmetic talc miners and millers with high exposures.

PCB 11 has been detected in air, water, sediments and human serum. PCB 11 has not been identified in any of the commercial PCB mixtures. Its presence in many cases is likely to be through products containing pigments in which the PCBs are inadvertently produced. Little information is available on the toxicity of PCB 11. The present study examines the effects of PCB11 on hepatic nuclear receptor activation in primary human hepatocytes. In addition, the effects of PCB 11 are compared to PCB77, 95, 126, 153 and Aroclor 1016 and 1254. Cryopreserved primary human hepatocytes (PHHs) cultures were exposed for 48 hours and subsequently measured for cytotoxicity, gene expression of CYP1A2, CYP2B6, and CYP3A4, and mRNA expression of TNF, IL8, NQO1 enzymes levels. Cryopreserved PHHs were exposed to chemicals at nominal concentrations of 0.2, 0.4, 0.9, 2.4, 9, 20, 40, 90, 200 μM (PCB 77, 126, 153 and Aroclors 1016 and 1254) or 2, 4, 9, 20, 40, 90, 200 μM (PCBs 11 and 95) or DMSO vehicle control. All concentrations were run in triplicate in three replicate plates. Cell viability was decreased at the highest concentration for all chemicals. All chemicals but PCB 95 induced CYP1A2 with a rank order of potency of PCB77 > PCB153 > PCB126 > Aroclor 1254 > Aroclor 1016. All chemicals except PCB126, a model Ah Receptor (AhR) activator, produced a 50-70-fold induction of CYP1A2. PCB11 only resulted in a 2-5 fold induction of CYP1A2 at concentrations of 90μM or higher, suggesting minimal AhR receptor activation. CYP3A4 was induced by all chemicals evaluated with a rank potency of PCB153 > CITCO > PCB95 > Aroclor1016 > PCY3A4 > PCB126. Aromatase (Arom) and CYP1A1 enzymes levels indicate these effects result in an increased risk of cancer. Analysis of animal studies that evaluated whether potential contaminants of cosmetic talc such as fibrous talc or other non-asbestiform elongate mineral fibers (regardless of dimensions) also did not support an increased risk of cancer. The animal studies support the premise that high doses of cosmetic talc and/or its contaminants could be associated with non-cancer respiratory effects, such as inflammation and fibrosis. These observed effects are consistent with the type of health effects observed in animal studies of other nuisance dusts, and human reports that have found evidence of talcosis in cosmetic talc miners and millers with high exposures.

The Cramer decision tree, published in 1978, is a procedure developed to estimate the toxic potential of a substance based on its chemical structure. Based on the structural functionalities present, a chemical is parsed into Class I of oral toxicological concern, Class II of intermediate toxicity and Class III where a presumption of safety cannot be assumed. The Cramer decision tree was incorporated into the Threshold for Toxicological Concern (TTC) approach for the risk assessment of chemical substances, in circumstances of relatively low exposure, for which toxicological data is not available. The TTC approach is used by both the European Safety Authority (EFSAS) and the World Health Organization (WHO) Joint Expert Committee on Food Additives (JECFA) in their evaluation of flavoring ingredients for use in food. In a 2016 workshop report, EFSAS and WHO noted that minor changes to the Cramer decision tree could reduce ambiguity and make it more adaptable to use within silico tools. With this in mind, the goal of this study was to develop an update to the original Cramer decision tree that reflects current metabolic and toxicologic knowledge, that expands the chemical space covered, but that also has a simplified structure relative to the original Cramer decision tree. The revised approach relies more heavily upon chemical structure-based questions to move compounds into classes. This approach enhances the ability of in silico tools to correctly apply the decision tree when assigning substances to various classes of toxicological potential. Toxicological poten-

Primary human hepatocytes (PHH) are the gold-standard for preparing in vitro 3D liver models but are limited by their scarcity and their vulnerability to freeze-thaw cycles. In recent years, the more readily available HepaRG cells (hepatoma progenitor cell line able to differentiate into hepatocytes) have shown to be more similar to PHH, showing comparable stable metabolic competence and inducibility, with the added advantage of data consistency over multiple thawing. HepaRGs are a novel liver 3D model consisting in encapsulated differentiated HepaRGS, able to make spheresoid within 5 days post-encapsulation. Viable over 5 weeks, they respond to various Cytochrome P450 (CYP) inducers (CYP1A2, 2B6 and 3A4), while maintaining ≥2-fold induction along their lifespan. We demonstrated by LC-MS/ MS the functionality of phase I enzymes (CYP1A2, 2D6, 2B6 and 3A4) by studying the hydroxylation of Phenacetin, Dextrometorphan, Bupropion and Midazolam. Furthermore, HepaRGs display polarity features as demonstrated by the efflux of fluorescent probe (CDFDA) in intercellular biliary pockets and by measuring hepato-specific functions, such as albumin and urea synthesis over 5 weeks. Using the radio-HPLC method, we next investigated the metabolism of different radiolabelled xenobiotics - testosterone (TE), 7-hydroxycon
marin (7-HC) and 7-ethoxycoumarin (7-EC) at increasing concentrations - by HepaRGs after 48h incubation. The detection of different TE metabolites proves the in vitro metabolic phase I functional key enzymes (CYP2C19, 2C9 and 3A4). Interestingly, HepaRGs were able to metabolize TE in 68-hydroxytestosterone without any saturation of the CVP involved in this process by contrast to 2D monolayer where a plateau was reached. Moreover, the metabolism of 7-EC and 7-HC demonstrated phase I and phase II (glucoroni
dation and sulfation) enzyme activity, respectively. In addition, we assessed the hepatotoxicity on HepaRGs with 6 drugs with known hepatotoxic potentials for acute (24h, 48h) or chronic (14 days) exposure and demonstrated their efficient prediction ability for toxic or non-toxic compounds. HepaRGs represent a stable and reproducible in vitro liver-mimicking tool for the study of compounds metabolism, drug-drug interactions and hepatotoxicity.

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The European Commission’s Cosmetic Regulation No. 1223/2009 (EU 2009) requires cosmetics marketed in the European Union (EU) to be safe for their intended use. EU regulations require each cosmetic formulation have a Product Information File (PIF), which includes a Cosmetic Product Safety Report (CPSR). A CPSR assesses toxicological endpoints for a cosmetic, including an evaluation of potential health risks of each cosmetic raw material, including impurities. A CPSR assessment evaluates each ingredient and impurity against a minimum of ten different toxicity endpoints, including acute toxicity, skin and eye irritation, skin sensitization, phototoxicity, genotoxicity, repeated dose toxicity, reproductive toxicity, and carcinogenicity, and assigns a point of departure (POD) for the overall risk evaluation of each chemical undergoing assessment. Ideally, the POD is the no-observed-adverse-effect level (NOAEL) from a repeated dose animal study that is 90 days or greater in length. Most ingredients and impurities in cosmetics have limited in vivo data for one or more of the toxicological endpoints, and the availability of toxicity data is further limited by the Cosmetic Regulation’s ban on animal testing. Therefore, application of data gap filling approaches is required to meet regulatory requirements. For example, despite a very limited dataset for the essential oil Litsea cubeba fruit oil, ToxServices was able to prepare a CPSR ingredient assessment by reviewing the literature and identifying citral, the primary constituent of the oil, as a surrogate to fill data gaps for the eye irritation, skin sensitization, phototoxicity, repeated dose toxicity, and reproductive toxicity endpoints. Ferulic acid (an antioxidant in skin care formulations) also has a limited dataset, and has no suitable surrogate with data to assess skin irritation, eye irritation, and skin sensitization endpoints. ToxServices employed a suite of modeling programs including Toxtree, VEGA, and OECD QSAR toolbox, to estimate toxicity and fill the data gaps for these endpoints. Examples and considerations for data gap filling approaches for the ten toxicity endpoints assessed in a cosmetic ingredient risk assessment will be illustrated and discussed, including use of read-across methods and goodness of fit indices to select chemical surrogates and assign a POD, suffi
ciency of in silico modeling to predict endpoints such as genotoxicity, and the importance of correct selection of methods validated by the European Centre for the Validation of Alternative Methods (ECVAM).

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To determine what chemicals can be inhaled during an everyday use of hair care products for African American women, 9 brands of products (4 hair relaxers, 6 shampoos, 6 conditioners, 2 conditioning treatments, 1 leave in condi
tioner, 1 hair masque, 6 hair lotions, and 1 hair serum) were analyzed for vol
tile organic compounds (VOCs). Each product was purchased at local beauty supply store and some came packaged as a kit. Only one of each type of prod
cut was tested; replicates were not purchased. Samples were analyzed by GCMS (gas chromatography mass spectrometry) with headspace sampling. To approximate what VOCs could be emitted upon use, samples were incubated at 37°C, and the vapor injected into the GCMS. Compounds were iden
tified by matching mass spectra to those in a chemical library (NIST). Since the purpose of this work was a screening to find VOC identities, concentrations of the VOCs were not determined. Over 100 compounds were detected and identified. They included terpenes / terpenoids (15), alcohols (12), hydrocarbons (57), chlorinated (3), hydrocarbons (57), ethers, diethers (6), esters (23) and aldehydes (5). Some compounds would not have been expected given the listed ingredients, and they include substances that are suspected carcinogens, for example 1,4-benzen, styrene, and benzene. Because mixtures of chemicals have more adverse health effects than individual components, preliminary experiments were conducted to test emissions from a subset of products for their toxicity. Four products were used: one hair relaxer, one shampoo, one conditioner, and one hair lotion. Each product was applied to a filter paper on the lid of the petri dish, and Escherichia Coli and Bacillus Subtilis were exposed to product vapors for 24-hours. These preliminary re
sults show no inhibition of bacterial growth.


Exposure data is a major challenge in the field of safety assessment process of cosmetic products. For many years, the European Scientific Committee on Consumer Safety (SCCS) has provided, through its Notes of guidance and opinions, a number of quantitative data about use frequ
cency, amounts of products applied, areas of application and expo
sure to some categories of cosmetics. However, all the data reported are related to the “Adult” exposure and no information about “realis
tic” exposure assessment to cosmetics in children from birth is available. In this context, the SCCS has stated to adjust the default assessment factor of 100 for children (from birth to 10 years old) by multiplying this factor by the difference in Skin Surface Area (SSA) over Body Weight (BW) ratio (SSA/BW) between adults and children. Thus, the difference between the SSA/BW ratio has been set at 2.3 for children from birth. Based on these elements, it seems interesting to check if this additional safety factor is sufficiently reliable to cover the exposure to cosmetics and their ingredients in children from birth. Through an exhaustive review of the exposure data available in literature for children from birth, a comparison has been performed between the P90 val
cues for children from birth versus adults for the same product categories. The exposure data have been chosen on the basis of relevant criteria such as: date of the study, type of sampling and analytic method, method of collecting data etc. Following this analysis, it has been found that some ratios calculated are higher than 2.3. Such results imply that the safety factor of 2.3 may not be sufficient to cover the exposure in children from birth for a number of cosmetic products. This difference highlights the importance of taking into account the most “realistic” exposure for some cosmetics instead of applying additional factor which may lead to an underestimation of the risk expected. The final goal of this work is always to ensure the safety of cosmetic products under normal or reasonably foreseeable conditions of use for all the populations.
Safety Assessment of Cosmetic Ingredients and Chemicals for Skin Sensitization Using QSAR In Silico Tool

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Skin sensitization methods are developed to protect workers and consumers from chemical exposures. These methods are well adopted in OECD 6 Test Guideline (TG) addressing 5 Key Events (KE) of the skin sensitization Adverse Outcome Pathway (AOP) such as 442C (Direct Peptide Reactivity Assay, DPRA); OECD TG 442D (Keratinosens™) and, 7 OECD TG 442E (human Cell Line Activation Test, h-CLAT). The two biomarkers based test SENS-15 and the Genomic Allergic Detection Test 10 (GARDs) are under consideration by the OECD for the development of the respective TGs. Here, our study aimed to test the practicability of in silico predictions using (QSAR) tools i.e. Toxtree and VEGA HUB to evaluate their use as a time- and cost-effective alternative to relate measured and calculated physical-chemical properties of chemical compounds to their sensitization potential. The 25 compounds have been selected from literature under non-sensitizer, weak, moderate, strong and extreme category with LLNA and Sens-15 prediction. These predictions are taken as benchmark for our QSAR analysis. VEGA predicted the skin sensitization potential of these compounds with 96% accuracy. However, Toxtree predicted the skin sensitization potential of 76% accuracy. The details, as well as forward and backward searches. Studies were selected for inclusion using PECO (Population, Exposure, Comparator, Outcome) criteria and evaluated for reporting quality, risk of bias, and sensitivity using a domain-based approach. Then each study was rated as high, medium, or low confidence. Evidence was synthesized and strength of evidence was summarized into categories of robust, moderate, slight, indeterminate, or compelling evidence of no effect, using a structured framework. Findings in the liver included changes in liver weight, changes in clinical chemistry, and histopathological findings suggestive of liver damage. The evidence for liver effects was considered moderate, based on consistent changes in liver weight in medium and high dose studies. The strongest evidence of an effect on liver enzymes and histopathological changes was observed in exposures to low doses of DEP over long durations; however, these studies were generally considered to be low confidence due to risk of bias concerns. Conversely, no histopathological effects were observed at higher doses in medium or high confidence studies. Differences in animal models, route, and study design between the lower and higher dose studies may contribute to the inconsistent pattern of findings. These conflicting findings suggest that additional research needs to be conducted on DEP following low dose exposures, which are more likely to be relevant to humans. The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the US EPA.

Use of Cross-Route Extrapolation to Develop an Oral Cancer Slope Factor for Isoprene and Its Application in a Drinking Water Risk Assessment

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According to the National Toxicology Program’s 14th Report on Carcinogens, isoprene is reasonably anticipated to be a human carcinogen. Although no reliable human epidemiology data are available, prolonged inhalation of isoprene causes tumors at multiple sites, including the liver, lung, pituitary gland, kidney, mammary gland, and Harderian gland in rats and/or mice. Isoprene can be found in drinking water as a result of leaching from rubber materials. Due to its volatility, few oral-route toxicity studies have been conducted. We performed cross-route extrapolation on benchmark dose modeling data that had served as the basis for a previously-published inhalation unit risk value to obtain an oral cancer slope factor (CSF) for isoprene. Cross-route extrapolation is justified because isoprene causes neoplastic and non-neoplastic effects at multiple distal sites; the most sensitive neoplastic effect is at a distal site (the liver); and measured and modeled toxicokinetic data are available to assess differences in route of exposure. Moreover, it is plausible that oral exposure to isoprene could result in a spectrum of neoplastic and non-neoplastic effects that is similar to what has been observed following inhalation exposure. We developed an oral CSF for isoprene using the lower bound EC10 (i.e., the LECO10 of 78.5 ppm (equivalent to 238.7 mg/m³) for liver tumor development in male mice as the point of departure. Using reference inhalation rate and body weight values for male B6C3F1 mice and a body weight ratio-based dosimetric adjustment factor, we converted the 238.7 mg/m³ LEC10 to a human equivalent dose of 1.42 mg/kg-day and then to an oral CSF of 0.0704 (mg/kg-day)⁻¹. We then used this oral CSF to identify an acceptable level (10⁻⁶ risk) of isoprene in drinking water. Because isoprene may act through a mutagenic mode of action, we applied age-specific drinking water ingestion rates to the oral CSF to obtain life-stage specific drinking water unit risk values, which were then summed across age groups to derive the total lifetime drinking water unit risk of 5.31 x 10⁻⁵ (μg/L)⁻¹. Applying this unit risk value to an acceptable cancer risk level of 10⁻⁶ yields an allowable concentration of 1.9 μg/L isoprene in drinking water.
Application of Toxicogenomics for the Risk Assessment of the Food Contaminant Acetamide

Michigan State University, East Lansing, MI; 1-Michigan Biotechnology Institute, Lansing, MI; 2-Scitovation, Research Triangle Park, NC; 3-Syngene International, Bengaluru, India; 4-Eurofins Advins, Bengaluru, India; 5-Exponent, Menlo Park, CA; and 6-Indiana University Bloomington, Bloomington, IN.

Acetamide (CAS 60-35-5) is detected in common foods including milk, eggs, beef, and roasted coffee beans. Chronic rodent bioassays using high doses (≥1000 mg/kg/day) suggest acetamide is a group 2B possible human carcinogen due to the induction of liver tumors. Weight-of-evidence indicates acetamide is not genotoxic, and therefore a threshold response is expected. We used a toxicogenomics approach in male Wistar rats gavaged daily for 7 days at doses of 30, 100, 300, and 1000 mg/kg/day (mkd) to determine the benchmark dose (BMD), and investigate its mode of action. No treatment related changes in terminal body weight, clinical chemistry, or histopathology were observed at doses of 30, 100, or 300 mkd. At 1000 mkd, the dose reported to elicit carcinogenesis, liver weights decreased 1.3-fold with the presence of single cell necrosis, hepatocyte vacuolization, and increased mitotic activity consistent with a 4.2 fold increase in the cell proliferation index. Accordingly, plasma ALT and AST were increased 2.0- and 2.2-fold, respectively. RNA-seq analysis identified 1 differentially expressed gene at 300 mkd, and 2,685 at 1000 mkd (fold-change ≥1.5 and FDR ≤ 0.05). Down-regulated genes were associated with lipid metabolism while up-regulated genes included cell signaling, immune response, and cell cycle functions. Collectively, these results revealed a no-observable adverse effect level (NOAEL) and no-observed-transcriptional effect level (NOTEL) at 300 mkd, warranting further investigation at doses between 300 and 1000 mkd to identify the benchmark dose (BMD). Function enrichment studies indicate that perturbations of cell cycle and lipid metabolism, though a more refined dose-response evaluation will be needed to demonstrate dose-dependent effects related to the MOA of acetamide. Funding by the Bill and Melinda Gates Foundation (OPP142801).

Sulfuryl Fluoride-Induced Neurotoxicity: Potential Direct Brain Access via the Intranasal Route


Sulfuryl fluoride is a fumigant registered to control structural and commodity pests such as termites, bedbugs, and the saw-toothed grain beetle. It kills insects by anoxia and interfering protein synthesis. As a consequence of ongoing evaluation of human health risks of fumigants, the California Department of Pesticide Regulation completed a risk assessment of sulfuryl fluoride in 2006 and an update to that risk assessment in 2018. The most current database includes registrant-submitted studies, open literature studies, and a physiologically based pharmacokinetic model in humans, acute inhalation exposure to high levels of sulfuryl fluoride causes respiratory irritation, pulmonary edema, renal injury, central nervous system depression, brain necrosis, and even death. Short-term or chronic inhalation exposures result in brain, respiratory system, dental, and kidney effects in both humans and laboratory animals. Fluoride is considered the active principal in sulfuryl fluoride-induced toxicity and it is commonly thought to work through either a systemic or portal of entry mode of action (MoA). Common neurotoxic effects in mice, rats, rabbits, and dogs were malacia (necrosis) and vacuole formation in the basal ganglia, also generally considered to have a systemic MoA. However, from our analysis of the updated toxicity database, we suggest that neurotoxicity may instead be mediated via direct entry through the nasal cavity, bypassing the blood-brain barrier. This novel MoA for sulfuryl fluoride neurotoxicity is supported by the following: 1) a fluoride brain-to-plasma (T/P) ratio for acute inhalation studies that is approximately 20-fold higher than T/P ratios from oral, intra-venous, or intraperitoneal studies with sodium fluoride (NaF); 2) sulfuryl fluo- ride-induced brain lesions are confined to the basal ganglia after inhalation exposure, but not after oral NaF exposure; and, 3) evidence of direct intranasal absorption for other chemicals. In conclusion, future studies should explore the possibility of a direct access to brain via the nasal epithelium, which would influence the calculation of reference concentrations.

Health Risks of Chemicals in Consumer Products: A Review

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Increasingly diverse chemicals are used in consumer products, while our understanding of their exposure pathways and associated human health risks still lags behind. This study aims to identify the dominant patterns of exposure pathways and associated health risks of chemicals used in consumer products reported in the peer-reviewed literature. We analyzed 342 articles covering 202 unique chemicals, and distilled the information on the functional use/product application combinations, including plasticizers, polymers/monomers, and flame retardants used in food contact products, personal care products, cosmetics, furniture, flooring, and electronics. We also published over the past decade, facilitating an updated MoA analysis for nasal tumors. These new data include analytical results discerning endogenous and exogenous biomarkers of exposure to formaldehyde and negative results from in vivo genotoxicity assays in target tissues of rats, as well as in genetically susceptible mice (including a recent NIEHS study conducted in p53-/- mice). Herein, we conduct a MoA analysis with special emphasis on studies published over the past decade. These data support a MoA involving exceed-
Antibiotic resistance (ABR) has become a global public health threat, as antibiotics used to treat diseases, including common infections, are increasingly becoming ineffective. Spread over 380 square kilometres in Himachal Pradesh’s Solan district, the Baddi-Barotwala-Nagarbar (BBN) industrial area is one of India’s largest pharmaceutical manufacturing hubs. The region hosts around 500 small, medium and large pharma units and accounts for 35 per cent of Asia’s total medicine production. But rapid industrialisation and a lax attitude towards safe disposal and management of pharma waste have raised concerns about the effects of pollution on the environment and health. Due to such practices, the BBN region remains prone to antibiotic pollution. They are among environmental persistent pharmaceutical pollutants which have not degraded completely during treatment. They may influence the genetic makeup of bacteria, leading to the survival of resistant bacteria and spread of antimicrobial resistance (AMR), a public health threat. Samples were collected from different area of BBN and they were identified for antibiotics resistant of bacteria in environment in laboratory. Antibiotics and resistant bacteria are present in the environment. Antibiotics could favour resistant bacteria. As in other environments, the significance of this process depends on the antibiotic concentration, its bioavailability and other constraints. This varies in water, sewage sludge, soils, and sediments, because the concentration of antibiotics, the physicochemical constraints and the mobility of bacteria as well as their resistance genes vary. The ability to take up DNA from the environment is widespread among natural isolates. To understand the interaction of antimicrobial compounds and bacteria in the environment and for a sound risk assessment, the use of test systems and field studies is crucial. For developing countries such as India, inadequate policy framework, limited stakeholder awareness, ineffective monitoring, limited focus on infection prevention and control, inadequate sanitation and hygiene, limited data on AMR surveillance and dearth of technology and resources have been the major challenges. Our study shows how improper disposal of pharmaceutical industry waste from Baddi, Himachal Pradesh, one of the largest pharmaceutical manufacturing hubs in India, could become a potential reason for emergence and spread of ABR.

Environmental stressors and contaminants can lead to Adverse Outcomes (AOs) in both human health and ecological receptors. Evaluating risks of AOs across multiple organisms in a community is crucial to characterize the cumulative impacts of stressors and contaminants; but is challenging due to species-specific differences in exposure pathways, behavior, physiology, and toxicity mechanisms. This work addresses some of these challenges by developing a quantitative source-to-outcome approach using the Aggregate Exposure Pathway (AEP) and Adverse Outcome Pathway (AOP) frameworks. We demonstrate this approach using a case study of a hypothetical site contaminated by a molecule that competitively inhibits iodide uptake into the thyroid at the sodium-iodide symporter (NIS), and TOP effects established AOPs. External exposure pathways were quantified in an AEP network describing chemical movement through the site. This network was used to predict NIS-inhibitor exposure for humans, fishes, and small herbivorous mammals under mild, moderate, and high contamination scenarios. Network analyses were applied to describe source apportionment for each species. Exposures were linked to an AOP network for NIS inhibition using physiologically based pharmacokinetic models, then combined with mechanistic dose-response data to calculate a hazard index (HI) for each potential AO in each species. The analysis predicted that surface water contamination was the largest contributor to source apportionment of exposure in fishes, while groundwater contamination was the largest contributor in humans and small herbivorous mammals. HI results predicted small herbivorous mammals to have the highest risk of AOs in all scenarios, while the relative risk of AOs in scenarios between humans and fishes differed by exposure pathway. This work demonstrates how the AEP-AOP construct can link environmental transport and transformation, exposure, toxicokinetics, and toxicodynamics; as well as inform cumulative risk assessment, by 1) organizing mechanistic data, 2) identifying data gaps, 3) quantifying uncertainties, and 4) facilitating simultaneous evaluation of risk in human health and ecological endpoints. The views expressed in this poster are those of the authors and do not necessarily reflect the views or policies of the US Environmental Protection Agency.
Using Evidence Mapping to Focus and Refine the Evaluation of Reproductive Endpoints in a Systematic Review of PCBs


Systematic review will often reveal a wide range of health outcomes associated with exposure to a chemical of interest. This is particularly true for chemicals that have thousands of studies available, such as polychlorinated biphenyls (PCBs). From a pragmatic perspective, a systematic review of all of these studies is not feasible. In such cases, it may be helpful to focus the systematic review on the outcomes identified as most impactful for protecting public health. Here, we describe the application of evidence mapping as an organizational principle for refining a large and complex database, using animal toxicology studies on the reproductive effects of PCBs as an example. A systematic review of PCB-related health effects identified >400 animal toxicology studies that evaluated reproductive toxicity. These studies were extracted into a literature inventory designed to capture relevant experimental design and health outcome details. The endpoints evaluated in each study were categorized according to type of outcome (e.g., fertility/fecundity, hormone levels, organ weights). To identify the most relevant adverse health outcomes to move forward for further evaluation, major considerations will include the number and types of studies evaluating each outcome, the severity or biological significance of each outcome, and the relative sensitivity of each outcome to PCB exposure. Once the most relevant adverse health outcomes are identified, studies reporting these outcomes can proceed through the systematic review process, where they will undergo study evaluation and further analysis. The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the US Environmental Protection Agency.

Identification of Points of Departure for Trimethylamine Exposure Guidance Levels

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Trimethylamine (TMA) is produced on a large-scale in the manufacturing of numerous products including dyes and herbicides and has been involved in several unanticipated releases. Toxicity information was used in the weight of evidence for identifying critical effects and points of departure (PODs) that could be used to derive exposure guidance values for TMA for 24-hour, 30-day, and 90-day durations of exposure. The highly alkaline corrosive nature of TMA is manifested by immediate portal of entry effects after oral or inhalation exposures. Longer-term systemic effects, produced by either oral or inhalation routes of exposure involve neurotoxicity and pathological changes in the liver and kidney. The respiratory tract is the primary target for inhaled TMA toxicity with endpoints ranging from sensory and pulmonary irritation, epithelial tissue damage, increased airway resistance, to lethality via pulmonary edema. Effect levels corresponding to the Acute Exposure Guideline Level (AEGL) 1, 2, and 3 Tiers respectfully, representing thresholds for mild/ reversible effects; serious/irreversible effects; and lethality, were identified. Inhalation POD values for the 24-h duration Tier 1 was 8 ppm from a human occupational study. Tier 2 was 918 ppm, used with an adjustment for severity, and Tier 3 was 918 ppm, the latter two levels derived from a 14-d rat study. All PODs were time-adjusted to 24-h following a C∞/t relationship. The POD for 30-d Tier 1 effects was 8 ppm, again from the human occupational study and the Tier 2 POD was 190 ppm from the 14-d rat study. A Tier 3 POD was not determined because of a lack of data. A 90-d Tier 1 POD used the previous human study while the 90-d Tier 2 and Tier 3 PODs could not be established due to a lack of relevant data. For oral exposures, in humans initial nausea and vomiting are followed by an initial inflammatory phase, with prolonged exposures resulting in necrosis with gastrointestinal and respiratory stenosis and stricture formation. The 24-h Tier 1 POD was 4.0 mg/kg-d, based on a human study, while the Tier 2 POD was 200 mg/kg-d, and the Tier 3 POD was 464 mg/kg-d based on rat studies. The 30-d POD values for Tiers 1, 2, and 3 were based on a rat developmental study, with values of 40, 200 and 200 mg/kg-d respectively. The 90-d Tiers 1, 2 and 3 PODs used 30-d data, subjected to a duration adjustment. These PODs can be used to develop exposure guidelines useful to the emergency response community and State and local public health departments in the case of unanticipated releases. This abstract may not represent the views and policies of the US Environmental Protection Agency.

Semi-automated Data Extraction Workbench for Environmental Health

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Systematic review, already a cornerstone of evidence-based medicine, has recently gained significant popularity in several other disciplines including environmental health and evidence-based toxicology. One critical and time-consuming process that must occur during systematic review is the extraction of relevant qualitative and quantitative raw data from the free text of scientific documents. The specific data types extracted differ among disciplines, but within a given scientific domain, certain data points are extracted repeatedly for each review that is conducted. To that end, Sciome has begun research and development of a semi-automated data extraction workbench for use in this context. We are focusing our research on three specific goals. First, we are using deep learning to build novel data extraction models to extract data elements of interest to those conducting systematic reviews in the domain of environmental health. Second, we are building a web-based data extraction software platform specifically designed for usage in the domain of systematic review. And finally, we plan to introduce new protocols to standardize the extraction process further for both humans and models, to help ensure that we report our results so far, including the performance of more than 20 novel data extraction components of relevance to environmental toxicology, created and tested on an annotated dataset from NTP. Performance varied widely among data types with some tasks inherently more difficult than others. For certain simple data types, like sex of the experimental animal, we achieved F-scores in excess of 95%; for more difficult entities, we were still often able to achieve an F-score of 65% or more, given sufficient training data. Because accurate data extraction can be a challenging problem, and given that current methods rarely achieve 100% accuracy, we are integrating our methods into a spell-checking system that combines machine and human intelligence in a manner that is superior to either in isolation. The system will: highlight extracted terms in a pdf; automatically populate extraction forms with extracted data; allow humans to intervene and correct the results; and learn from the corrections to continually update the model. The resulting system will meet both systematic reviewers and modelers’ needs more efficiently and less expensively to maintain, greatly accelerating the process by which scientific consensus is obtained in a variety of health related disciplines having great public significance.

The Escalating Severity of Boron Trifluoride Inhalation Effects Necessitates Prompt Emergency Response Actions

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Boron trifluoride (BF3; CASRN 7637-07-2) is an industrial catalyst existing as a colorless gas with a readily detected odor. It is also used as a fire retardant and in various nuclear science applications. NASA estimated that 50,000 workers are potentially exposed annually; industrial and transportation accidents can result in additional unanticipated and uncontrolled exposures. Toxicity at both the portal of entry and systemic tissues occurs following inhalation exposures. High concentrations cause acute lethality from direct pulmonary effects, while prolonged exposure to lower concentrations can cause lethality from renal effects. Inhalation studies covering durations to 13 weeks demonstrate concentration responses for pulmonary and renal effects, and lethality. Acute Exposure Guideline Level (AEGL) values are available for up to 8 hours, but no values exist for longer durations. BMD 50 and BMD 05 values for 4-h lethality differed by only 1.5-fold. Because inhalation exposures may persist beyond 8 hours and the concentration-response for lethality is steep, data for multiple effects for durations beyond 8 hours were examined in the context of AEGL tiers 1, 2 and 3, representing thresholds for mild and reversible effects; more serious irreversible or escape-impairing effects; and lethality, respectively. Tiered points of departure were discriminated for acute exposures (ranging 4-fold) by no effect levels, respiratory irritation and death; for short-term exposures (ranging 8-fold) by urinary biomarkers, organ weight changes and death; and for subchronic exposures (ranging 8-fold) by urinary biomarkers, renal histologic changes and death. The narrow range of POD values for these effects further underscores the need for prompt characterization and control of exposures from unanticipated BF3 releases. This abstract may not represent the views and policies of the US Environmental Protection Agency.

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An Integrated Approach to Evaluate Common Mechanisms of Toxicity in Pesticides to Support Cumulative Risk Assessment


In 2016, the United States Environmental Protection Agency’s (US EPA) Office of Pesticide Programs published a guidance framework for establishing candidate common mechanism groups (CMGs) and conducting cumulative risk assessment (CRA) weight-of-evidence-based screenings. A candidate CMG is a group of chemicals that share characteristics such as structure, apical endpoints, and mechanistic data that indicate a common mechanism of toxicity may exist. Although a candidate CMG lacks sufficient data for establishing all key events in an adverse outcome pathway, it requires less data to be established than a full CMG and, as such, it allows the US EPA to more efficiently screen pesticides for CRA purposes. In the current study, a multi-tiered approach was used to investigate mode of action through literature review, and to analyze in vivo toxicity data, in vitro Toxicity Forecaster (ToxCast) data, chemical structural features, and physicochemical properties of dinotroaniline pesticides currently registered in the United States. This analytical approach was used to determine whether nine dinotroaniline compounds or a subset of those listed compounds may form a candidate CMG. The pesticidal mode of action for dinotroanilines involves disruption of root and bud microtubules of plants. Despite a high degree of structural similarity among at least three dinotroanilines and shared target organs that were identified for some of the chemicals based on effects observed in in vivo studies, there were no consistencies among the groups, suggesting lack of a common mechanism when all available data were considered together. For example, two compounds with a high degree of structural similarity and thyroid/liver in vivo effects were not found active in any ToxCast assays. Everything considered, the weight-of-evidence is insufficient to support the testable hypothesis that dinotroanilines could form a candidate CMG, and highlights the importance of establishing a consensus among multiple analyses prior to CRA. Disclaimer: The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the US EPA.

Application of a “One-to-Many” Analog Approach with Consideration of Toxicokinetics in Consumer Product Safety Assessments

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A “one-to-many” read-across approach, in which data from a tested compound is read across to multiple structurally-related untested, target compounds, can be used when for risk assessment of structurally and functionally related chemicals. Using a case-study of octylphenyl glycol ether (OGE) surfactant ingredients in several consumer products, including cosmetics and detergents, data relevant to 4-tert-octylphenol (4-OP) was read-across to two other OGEs, which are structurally identical to 4-OP except with three to six ethoxy units in place of the hydroxyl group. 4-OP is weakly estrogenic, binds to the estrogen receptor, and has been shown to result in various reproductive effects at doses exceeding the metabolic saturation threshold at doses between 50 and 200 mg/kg/day. Crucially, the presence of the ethoxy chain in the target compounds is anticipated to reduce the toxic potency of the OGEs compared to the source compound, 4-OP. Twenty-four repeated dose and reproductive toxicity studies on 4-OP were evaluated, and seven were deemed reliable for assessment. The key study selected for the point-of-departure (P0D) was a two-generation reproductive feeding study in rats, in which exposure to 240 mg/kg/day of 4-OP resulted in an 18% reduction in relative uterine weight and 16% decrease in the mean body weight of the F1 pups. The corresponding human-equivalent non-human equivalent effect level (NOAEL_{h}) of 5.1 mg/kg/day was the lowest P0D among the seven studies considered, with the exception of a four-week repeated dose study that was potentially confounded by gavage-specific irritative effects. Few published risk assessments in the literature utilize a one-to-many read-across approach and relatively little guidance is available for doing so. Here, we have shown one way in which the one-to-many approach could proceed, in which it can be clearly shown that the source compound is likely to be more toxicologically potent than all the source compounds. Such an approach is likely to be health-protective, but overly-conservative.

Derivation of a Total Allowable Concentration for Methyl Acetate

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Methyl acetate (MeOAc) is a volatile solvent that has been detected as a leachate from drinking water systems. This poses the potential for a total allowable concentration (TAC) for MeOAc in drinking water following NSF/ANSI Standard 61 (Drinking Water Components: Health Effects) risk assessment guidelines. NSF/ANSI Standard 61 Standard 61 defines a TAC as the maximum concentration of a nonregulated contaminant allowed in a public drinking water supply. MeOAc has low acute oral toxicity. Acute inhalation exposure in humans may cause transient narcotic effects, blindness, and optic nerve damage. No animal repeated-dose oral toxicity studies were identified. A 28-day inhalation study in rats identified a NOAEC at 1,057 mg/m³ and a LOAEC at 6,040 mg/m³ based on impaired body weight (BW) gain, decreased food consumption, and pathological changes in the olfactory epithelium. MeOAc is not mutagenic or clastogenic based on negative results in two bacterial reverse mutation assays, and a micronucleus assay in rats. The US EPA evaluated the toxicity of MeOAc in a health and environmental effects profile published in 1986 and a provisional peer-reviewed toxicity value document published in 2010. Due to the lack of oral repeated dose toxicity data for MeOAc, US EPA relied on data for methanol (MeOH), the immediate in vivo hydrolysate product of MeOAc, to evaluate MeOAc’s toxicity in both documents. In 2010, US EPA established a screening peer-reviewed r-PID of 1 mg/kg/day for MeOAc by adjusting the MeOH r-PID of 0.5 mg/kg/day by the molecular weight (MW) ratio of MeOAc and MeOH. The MeOH r-PID of 0.5 mg/kg/day was derived using an oral NOEL of 500 mg/kg/day based on changes in serum chemistry and decreased brain weights in 90-day oral rat studies, and adjusted by a composite uncertainty factor of 1,000 (10 for interspecies extrapolation, 10 for intraspecies extrapolation, and 10 for subchronic to chronic extrapolation). However, in 2013, the MeOH r-PID was updated from 0.5 mg/kg/day to 2 mg/kg/day, based on an oral NOEL of 500 mg/kg/day after changes in serum chemistry and decreased brain weights in 90-day oral rat studies, and adjusted by a composite uncertainty factor of 1,000 (10 for interspecies extrapolation, 10 for intraspecies extrapolation, and 10 for subchronic to chronic extrapolation). In 2015, the MeOAc screening r-PID of 1 mg/kg/day is outdated. New toxicity data that would impact the US EPA’s assessments of MeOAc or MeOH was identified. Therefore, using US EPA’s r-PID for MeOH to calculate an r-PID for MeOAc is still appropriate. ToxServices derived an oral r-PID of 4 mg/kg/day for MeOAc by adjusting the data to support the use of a 20% relative source contribution factor, a BW of 70 kg, and 2 L/day drinking water intake, results in a TAC of 30 mg/L for MeOAc.

Comparison of Dam Toxicogenomic and Dam/Embryo-Fetal Apical Points of Departure in a Rat Ketoconazole Developmental Toxicity Study

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Toxicity testing paradigms for novel molecules take several years of investment and require extensive animal usage. There is promise that leveraging alternative techniques, such as toxicogenomics, may be able to replace traditional testing strategies for risk characterization while reducing animal use and maintaining protection for human health. The objective of this study was to generate in vitro data to support the use of a transcriptome benchmark dose (BMD)-based point of departure for developmental and reproductive toxicity (DART) by comparing transcriptome to traditional apical points of departure. Ketoconazole, a fungicide with established embryotoxic and fetotoxic properties, was used for proof-of-concept in a rat developmental toxicity study. Ten rats/dose group were exposed daily to vehicle control or ketoconazole with seven dose levels ranging from 0.63 to 40 mg/kg/day from gestation day (GD) 6-21. On GD 21, embryotoxicity and fetotoxicity was evaluated with standard endpoints from the OECD 414 guideline, and whole-genome long RNA (mRNA) sequencing was performed on dam liver and placenta samples. Lower confidence limits of BMDLs (BMDLs) were generated on apical endpoints using BMDS software and normalized gene expression data using BMDEXpress2.2 software. Dam apical BMDLs ranged from 0.42 to 13.88 mg/kg/day, with placenta histopathology as the most sensitive endpoint. Fetal apical BMDLs ranged from 13.97 to 20.35 mg/kg/day, with postimplantation loss as the most sensitive and stenogene delayed ossification as the least sensitive endpoints. Dam liver and placenta pathway-level BMDLs were 1.0 and 1.8 mg/kg/day, respectively. These BMDL values were roughly ten times more sensitive than any embryo-fetal toxicity apical endpoint evaluated in the study but more closely approximated the most sensitive dam apical endpoint BMDL (placenta histopathology at 0.42 mg/kg/day). These
data support the concept that labor- and animal-intensive DART studies could potentially be replaced with streamlined studies that employ transcriptomic data to identify a point of departure (in theory utilizing the liver as a sentinel organ), which would not only be protective of human health but also reduce animal use.

**2766 The Utility of Informative Prior in Benchmark Dose Modeling for Animal Reduction**

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The benchmark dose (BMD) method has a number of important advantages over the traditional NOAEL/LD50 (no/lowest observed adverse effect level) approach, however, the substantial potentials of the method have been considerably limited by prevailing dose-response modeling strategies and practice. The recently developed Bayesian benchmark dose (BBMD) modeling framework not only provides a useful tool for probabilistic dose-response assessment, but also offers an opportunity to reduce animal use in experimental testing while maintaining the similar accuracy in BMD estimation. This study focuses on investigating how empirical informative prior (IP) derived from toxicological data in regulatory risk assessments may impact the accuracy of BMD estimated using various animal sample sizes. Commonly used dose-response models (e.g., Logistic, Multistage) were fit to simulated dose-response data generated with different sample size from a few assumed “true” dose-response curves with different shapes. Such simulation study design allows us to explore situations where the empirical IP may or may not be compatible to the true dose-response relationship. How likely the estimated confidence interval (CI) of BMD will cover the true BMD and the width of the CI were estimated from datasets with a typical toxicological study design (e.g., four doses with 50 animals in each level) using uninformative prior, and compared with the counterparts estimated using empirical IP with reduced sample size. Results show that more than 40% reduction in animal use can be achieved when the IP is compatible with (i.e., having similar DR shape as) the “true” curve. However, the accuracy of BMD estimates can be impaired due to the limited flexibility of model shape imposed by the IP when the compatibility is weak. The results highlight the importance to use toxicologically based informative prior in dose-response assessment.

**2767 Assessing Somatic and Psychosocial Health Risks of Water Body Contamination on Human Communities in the Niger-Delta, Nigeria**

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Researches exist on the environmental consequences of oil extraction. The somatic and psychosocial health risks of Water Body contamination on humans in the Niger Delta region of Nigeria have however not received adequate research attention. 226 participants, purposively drawn from five Niger Delta communities, responded Water Body Contamination Scale (WBCS), General Health Questionnaire (GHQ-12) and Mental Health Continuum-Short Form (MHC-SF). Perceived prevalence of the effects of the domains of Water Body Contamination (WBC) are Nature/Environment Risk (NER) 54.0%, Life Style Disruption (LSD) 50.9%, Physical Health Risk (PHR) 53.4%, Mental Health Risk (MHR) 52.6%, Perceived Ability to Control Effects (PACE) 37.2% and WBC 58.6%. Observed prevalence of severe psychological distress include inability to concentrate (17.3%), insomnia (13.6%), feeling not useful to society (5.5%), confusion/indecision (2.8%), being constantly under strain (7.3%), inability to overcome difficulties (18.3%), do not enjoy normal activities (5.5%), inabil-

**2768 Systematic Review and Literature Mapping of Naphthalene Adverse Health Outcomes**


Since the posting of the 1998 naphthalene assessment by the US EPA’s Integrated Risk Information System (IRIS), the human and animal study naphthalene literature base has been significantly updated. The goal of this project is to evaluate the new evidence to help guide the research assessment to focus on the appropriate health outcomes. It is important to evaluate the new evidence across evidence streams for evaluating biological plausibility and human relevance on naphthalene-induced toxicities. A systematic review and literature map was used to summarize the literature characteristics in broad categories of evidence streams and health outcomes. Firstly, a broad-based literature search was conducted utilizing four online databases (PubMed, Web of Science (WOS), Toxline and Toxic Substances Control Act Test Submissions (TSCATS)) and additional search strategies that identified 15,935 studies. An initial electronic screen followed by title/abstract and full text screening identified 129 relevant citations based on Populations, Exposures, Comparators, and Outcomes (PECO) criteria that were mapped to human health (n=31), animal toxicology (n=86), and physiologically-based pharmacokinetic models (n=10) studies. Health outcome mapping revealed that the top five human health outcome categories included reproductive (n=9), immunological (n=7), hematological (n=3), hepatic (n=3), and endocrine/exocrine (n=3) systems. Mapping of the animal toxicology literature identified hepatic (n=22), immunological (n=20), reproductive (n=13), renal (n=12), and immunological (n=12) systems as the top five health outcome categories. These evidence maps are critical for prioritizing study evaluation and data extraction efforts that shape the evidence synthesis and integration, and dose-response analysis needed for health assessment.

**2769 Development of Aggregate and Cumulative Risk Assessment System of Hazardous Substances in Agricultural Environment in South Korea**


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Establishment of an ongoing monitoring in agricultural environments and rapid risk assessment system of hazardous substances for human would make the risk management in agricultural environment more efficient. In South Korea, a systematic approach started with the need of rapid and accurate risk assessment, which might take advantages of using even national-wide big data on hazardous substances. Agricultural Products Risk Assessment System (APRAS) developed by National Institute of Agricultural Sciences (NIAS) has been set up to evaluate human exposure and risk based on the residual monmoranic and hazardous substances in the agricultural environment, such as pesticides, heavy metals, and environment persistent pollutants in the agricultural environment. APRAS deals with aggregate assessment that includes all assessable pathways to the substance-specific exposure, and cumulative assessment of the group of substances with the same toxicity mechanism. In addition, it covers probabilistic evaluation function to reflect the national characteristics of Korean population. As a result of evaluating the aggregate and cumulative risks for dioxins with the same toxic mechanism through APRAS, it was confirmed to be very safe not only for the whole Korean adults but also for infants. The results of this system has been mutually verified with the results from the commercial probabilistic evaluation model and other risk assessment models developed in the developed countries, which can ensure the reliability of the system. APRAS can be applied to establish research directions or present policy proposals for more efficient management of hazardous substances in agricultural environment, for example, setting up the criteria of agricultural products or soil remediation.
Benzo(k)fluoranthene (BkF) and indeno(1,2,3-cd)pyrene (IP) are two polycyclic aromatic hydrocarbons (PAHs) that have extracted from several consumer products. Both PAHs are classified by US EPA (2010) as probable human carcinogens (B2), based on no human cancer data and limited animal data, and are on California’s Proposition 65 List. In order to develop No Significant Risk Levels (NSRLs) for these compounds, a relative potency factor (RPF) approach was utilized. The method involves calculating the relative potency factor (IP/BkF) as the ratio of carcinogenic potency of the compounds containing chemicals listed on Proposition 65 must include these chemicals on a warning label. However, Proposition 65 chemicals may be exempt from these requirements if a discharge or exposure is below the chemical's respective.

The US EPA and OEHHA have selected BaP as the index PAH because it has a robust toxicological dataset, including numerous rodent studies in which the carcinogenic potencies of BaP are shown to be equal to or greater than those of other PAHs. The US EPA determined cancer potencies of 3 and 7% for BaP and IP, respectively, compared to BaP. These potencies are based on positive results in rat lung implantation studies. OEHHA has derived a NSRL of 0.06 µg/day for BaP using an oral cancer slope factor of 1.2 x 10^-11 mg/kg-day^1 derived from gastric papillomas and squamous cell carcinomas in Swiss Webster mice fed 50-200 ppm BaP for 4-6 months. Therefore, screening-level NSRLs for BaP and IP were derived by applying RPFs for BaP and IP to the NSRL for BaP. The equation used to calculate screening-level NSRLs for PAHs is: BkF NSRL / PAH RPF = PAH NSRL. Applying the RPF of 0.03 for BkF gives a NSRL of 0.06 µg/day / 0.03 = 2 µg/day. Applying the RPF of 0.07 for IP gives a NSRL of 0.06 µg/day / 0.07 = 0.85 µg/day. Screening-level NSRLs for BaP and IP can be used in a safe harbor assessment to evaluate whether products that contain these PAHs may be exempt from California Proposition 65 labeling requirements.

The federal Agency for Toxic Substances and Disease Registry (ATSDR) released a 3-compartment Shower and Household Water-use Exposure (SHOWER) model (v1.0) in May 2018 that estimates inhalation and dermal exposure to chemicals in household water. The model predicts exposure for various showering and bathing schedules, including contaminants from other sources, such as dishwashers, clothes washers, toilets, and faucets. Model outputs include average daily human exposure concentrations and dermal doses for households with 1, 2, 3, and 4 persons. The model predicts air concentrations in a shower stall, bathroom, and main house throughout the day and accounts for chemicals entering a compartment either directly from sources within the compartment or indirectly from other compartments. Exposure is calculated by tracking contaminant air concentrations in relation to the activity pattern of the person in the house. Results are provided for the most highly exposed person in the household, usually the person taking the last shower in the home. For example, for a scenario consisting of a combination of multiple showers and carbon tetrachloride in water at 100 ppb, the model predicts average daily human exposure concentrations of 8, 15, 20, and 25 µg/m³ for households with 1, 2, 3, and 4 persons, respectively. The predicted concentrations are below ATSDR’s Minimal Risk Level and US Environmental Protection Agency’s (US EPA) Reference Concentration for carbon tetrachloride. The model-predicted dermal doses range from 0.37 µg/kg/day for adults to 0.68 µg/kg/day for children aged 1 to 2 years and are below US EPA’s Reference Dose. ATSDR used experimental data from US EPA to verify the model. The simulated concentrations are in good agreement with the experimental data considering the complexity of the model and the uncertainty associated with the experimental data. Using methods pioneered by Julian Andelman, Tom McKone, and others, the ATSDR SHOWER model advances the science of and provides a convenient platform for assessing inhalation and dermal exposure from contact with contaminated household water. Advantages of using the model include 1) assessing exposure for households with up to four members, 2) evaluating exposure from being home all day or away 10 hours, and 3) including other household activities that use water. The SHOWER model is available as a desktop application upon request at showermodel@cdc.gov.

Deriving human health risk estimates for environmental chemicals has traditionally relied on toxicity data from humans and/or experimental animals. In the absence of in vivo toxicity data, new approach methods (NAMs) such as read-across have the potential to fill regulatory data gaps. This case study applied an expert-driven read-across approach to assist in screening-level assessment of non-cancer oral toxicity for p,p'-dichlorodiphenyldichloroethane (p,p'-DDD), a data-poor chemical known to occur at contaminated sites in the U.S. Three potential analogues with existing non-cancer oral reference values were identified and evaluated for suitability based on three primary similarity contexts (structural, toxicokinetics, and toxicodynamics). Furthermore, in vitro high-throughput screening (HTS) assays from ToxCast were evaluated for evidence in support of the similarity justification for read-across. Analysis of ToxCast assay results revealed similarities in the bioactivity profile of the target, p,p'-DDD, which argues with a relative potency factor (RPF) of 0.07 for IP, demonstrated values (e.g., dose-effect relationships) in rat pulmonary systems, which is a potential influence of metabolism in the detoxification/bioactivation of environmental chemicals are often unaccounted for in in vitro systems, and should be considered in the interpretation of ToxCast assays when evaluating analogues for read-across. The views expressed in this abstract are those of the author and do not necessarily reflect the views and policies of the US EPA.

Characterization of safe use for spray and aerosol products includes the development of physical characterization and generation rate data, exposure estimations with consideration to chemical physical properties, and identification or derivation of safety thresholds for data-rich and data-poor chemicals for systemic and local endpoints. Multiple risk assessment techniques and tools are required to achieve confidence in a comprehensive safety conclusion. These include tools for exposure estimation (e.g., ConsExpo) and demonstration of safety using (Q)SAR to identify structural alerts and Cramer class with application of the Threshold of Toxicological Concern (TTC) for data-poor chemicals, specified or derived No Expected Sensitization Induction Level (NESIL) values to identify Acceptable Exposure Limits (AELs) for dermal sensitization, and reference to systemic or Low Observed Adverse Effect Levels (NOAEL or LOAEL) identified or derived through published literature search. A framework approach is presented (that highlights methodologies with lower confidence to inform future research needs) using case studies for common fragrance ingredients (e.g., linalool, β-pinene, and isoeugenol with maximum use rates of 1%, and the minor ingredient isobutyl methacrylate estimated at a maximum of 0.018%) and formulary components (e.g., dipropylene glycol and ethoxylated lauryl alcohol at maximum use rates of 10%). Overall, total estimated local and systemic exposure using default parameters resulted in Margin of Safety (MOS) > 1 over the Acceptable Daily Intake (ADI), AEL, or TTC, and Margins of Exposure (MOE) > 100 over NOAELs for all components except for α-pinene, where MOE values of 5.3 and 2.2 for adults and children, respectively, were identified based on a NOAEL of 0.36 mg/kg-day identified from a recent (2016) 90-day inhalation study in rats with a LOAEL of 25 ppm (LOAEL, γ = 3.6 mg/kg-day), thus indicating a recommended maximum use level of 0.02% within the context of this evaluation. Product specific rates and fraction of generated airborne particulate would further refine the assessment.
Rapid Evidence Mapping (rEM) (Lam et al. 2018) has been developed as a resource-efficient approach for rapidly compiling a high-level view of the available evidence using rigorous, transparent and explicit methodological approaches. Here we have applied the rEM approach to a case study mapping the evidence available with respect to possible adverse effects of feminine care products (FCP). This exercise illustrates how rEMs can be quickly created with the assistance of software tools using a much-reduced level of effort compared to what is typically required to produce a detailed evidence map or scoping study. Specifically, we examined the categorizations of included studies, binned by FCP type, biological exposure, and health outcome and the intersections of these categories. The resulting evidence map was developed to: identify areas where the most research has so far been conducted; identify potential evidence gaps where more research is needed; and target areas where sufficient information may exist for pursuing a systematic review. We identified 16,425 abstracts from a PubMed search and screened references in SWIFT-Active Screener, utilizing the software’s built-in machine learning model that identified 13,241 papers. Of these, 94% were relevant. Among the remaining 12,622 references, Active Screener software predicted that <6% would be relevant; this was confirmed with a small amount of additional screening. After screening was complete, 1,309 relevant studies were ultimately included. We identified research areas having a large body of evidence where a follow-up review, such as a systematic review, may be informative (e.g., human exposures to staphylococcus and toxic shock) as well as areas where literature is lacking (i.e., biological exposure linkages with sprays, wipes, intravaginal washing, herbal product types; ingredient linkages with douches, herbal medicine, sprays, sanitary pads; and general linkages between FCP types and health outcomes). The resulting Rapid Evidence Map provides a useful tool that can be used to quickly review the evidence base for multiple decision-making points and serve to illustrate how, when combined with the expertise of a review team, rEMs can provide an efficient form of knowledge synthesis.

**2775 Benchmark Dose (BMD) Modelling Analysis of In-Field Data of Human Respiratory Epithelium (MucilAir) to Establish a Toxicological Point of Departure (POD)**

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The utility of conventional in vivo animal studies to characterize toxicity associated with repeated inhalation exposures to irritants is limited by the complexity of extrapolation to humans, as well as by animal welfare concerns. An in vitro model of the human upper airway epithelium (MucilAir™) is a functional model of the human respiratory tract and accurately represents potential effects in vivo. This model was used in the evaluation of human toxicity for an irritant chemical. A number of endpoints were assessed, including Trans-Epithelial Electrical Resistance, Lactate Dehydrogenase Release and Resazurin metabolism which serve as markers of the surface irritant effect. Ten exposure concentrations ranging from 2 and 200 mg/L were evaluated in human respiratory epithelial cells as six replicates from five donors. A Hill model was fitted to the data using the BMDS software developed by the US EPA. Our assessments of model fit strongly favoured the Hill model over alternative polynomial models. Different definitions of the benchmark response (BMR) with various values of the benchmark response factor were evaluated, including the relative change BMR of 10% in the mean response over the control group, the one standard deviation BMR and the point BMR. The estimated Hill models well captured the patterns of dose-response curves. The BMD estimates were comparable across donors and endpoints, and their ranges and the 95% lower limits were used as the POD to determine chronic inhalation risk assessment values. Assessment of this POD against tissue-level exposure values demonstrated the safety of the assessed uses of this compound. This in vitro model is a fit for purpose alternative method for quantitative risk assessment of inhalation exposure to irritant materials.

**2776a Fipronil Hazard Identification for Occupational and Residential Exposure**

**2776 Comparison of Lung Cancer Risks from Environmental Exposures to Arsenic and from Those Associated with Medical Monitoring Criteria for Smokers**


The Agency for Toxic Substances and Disease Registry (ATSDR) may request medical monitoring as a follow-up to health evaluations at contaminated Superfund sites. Despite the general medical monitoring criteria outlined by ATSDR, it is sometimes difficult to determine whether potential disease risks among individuals who might have been exposed to specific environmental contaminants (e.g., arsenic in soil or water) at or near Superfund sites may warrant medical monitoring. In contrast, detailed criteria for lung cancer screening based on smoking history are available (i.e., lung cancer screening is only recommended for older individuals who have smoked in the past 15 years with at least 30 pack-years of cumulative smoking). Here, we present a case study comparing theoretical lung cancer risks from exposures to arsenic in drinking water and smoking, eligible for medical monitoring. We first calculated lifetime excess lung cancer risks for adults and children using a hypothetical arsenic concentration in soil, applying exposure assumptions used to derive the United States Environmental Protection Agency (US EPA) residential soil Regional Screening Levels, as well as more plausible exposure assumptions, and an oral lung cancer-specific cancer slope factor (CSF) for arsenic of 1.6 and 2.1 per mg/kg-day, for men and women, respectively. This CSF assumes a linear dose-response relationship, although evidence supports a threshold. Assuming an arsenic in soil concentration of 225 mg/kg and US EPA default exposure assumptions, the hypothetical lifetime excess lung cancer risks were 3.3 x 10⁻⁴ (0.03%) and 4.5 x 10⁻⁴ (0.05%) for men and women, respectively. For calculating the cumulative lung cancer risks of heavy smokers eligible for lung cancer screening, we relied on published absolute and relative lung cancer risks estimated from nearly a million men and women in the US during 2000–2010. Considering age- and sex-specific absolute lung cancer risks, relative lung cancer risks specific to smoking behaviors and history, and assuming various ages at which individuals started smoking, we calculated excess lung cancer risks due to smoking to be at least 2.8% and 1.6% for men and women, respectively. In conclusion, potential lung cancer risks from exposure to arsenic in soil at a concentration of 225 mg/kg are at least 3-fold lower than those of lower smokers if US EPA default exposure assumptions are applied, and potentially up to 200-fold lower if more plausible exposure assumptions are applied and, thus, do not warrant lung cancer screening.

**Fipronil Hazard Identification for Occupational and Residential Exposure**


Fipronil is a phenylpyrazole insecticide that blocks gamma-aminobutyric acid-gated chloride channels leading to central nervous system excitation and death. It is used to kill ticks and fleas on companion animals and for public health-related pest control. Clinical symptoms of exposure include seizures, dizziness, sweating, nausea, vomiting, diarrhea, blood pressure, eye and throat irritation, headache, coughing, and tremors. In acute toxicological studies in rats, mice and rabbits, fipronil caused convulsions, seizures, tremors, gait abnormalities and lethargy. In chronic studies, fipronil targeted the liver, thyroid and kidney. It is oncogenic in rats (thyroid) and mice (liver). In rats, over 80% of orally administered fipronil is absorbed; a significant portion is reabsorbed via enterohepatic circulation. Fipronil has a high log Kow partitioning into fat, and is expected to bioaccumulate. Dermal absorption is ~4.3%. The critical acute oral no observed effect level (NOEL) of 0.02 mg/kg/day was derived from a combined rat chronic toxicity-oncogenicity study. A statistically significant, 25% decrease in serum thyroxine (T4) occurred in males by Day 7 at the lowest observed effect level (LOEL) of 0.06 mg/kg/day. The critical acute oral NOEL was used for acute dermal and inhalation routes, as T4 (the most sensitive endpoint) was not monitored in route-specific studies. The critical subchronic and chronic oral NOEL of 0.02 mg/kg/day was based on sustained T4 decreases, convulsions, and death at the LOEL (0.06 mg/kg/day) after 23-50 weeks. Fipronil induced thyroid tumors in rats at high doses (13-17 mg/kg/day) that resulted from increased hepatic clearance of T4, leading to activation of the hypothyalamic-pituitary-thyroid axis. This mechanism is only relevant in rats, as humans have tighter T4 buffering. As such, a risk calculation based on T4 effects would presumably protect against thyroid tumors in humans. There have been numerous reports of adverse human health effects following fipronil exposure. These reports, coupled with concerns about in utero T4 deficiencies that could lead to mental retardation, hearing loss, and bone growth deficits -- and what role fipronil exposure may play in the development of these effects prompted a comprehensive human health...
A Review for the Best Practices for the Application and Preparation of SEND Data Sets

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Since December 18, 2016 Standard Exchange of Nonclinical Data (SEND) data sets have been required for New Drug Applications (NDA), abbreviated new drug applications (ANDA), and certain biologics license applications (BLA) and since December 18, 2017 SEND was required for nonclinical data contained in all Investigational New Drug Applications (INDAs). The preparation of these data sets is an additional step in the process for submission. As always timing is crucial for submissions with sponsors looking for the most aggressive timelines to reach the market and gain market competitiveness. This article will look at the key steps to optimize delivery of these SEND data sets. This optimization starts with alignment of terminology and the internal scheduling and processing; so that the draft and final SEND data sets can be sent to the client within days of issuance of the definitive reports. The internal processing and scheduling works too, for legacy studies or studies run by a client at a different CRO. The key factor here is effective and proactive communication with the Sponsor to define the format of the data and thereby reduce or minimize the need for any data re-entry or manual manipulation of data. This presentation will illustrate the processes for formatting send data sets based on experiences learnt to date when preparing SEND data sets for US FDA submission, and looking forward March 2019, some Safety Pharmacology will be included, with SENDIG3.1.

Recent Insights into the New ECHA/EFSA Endocrine Disruptor Guidance


New European Union (EU) Guidance on evaluation of chemicals for endocrine disruption was published in June 2018. This is particularly applicable to pesticides and biocides since for these, endocrine disruption is (under legislation) a hazard-based exclusion criterion, and therefore a major regulatory hurdle. The Guidance requires a comprehensive documentation of all endocrine-related measurements, across all studies including published literature, is notably time-consuming and prone to input error. The minimum base dataset for a sufficient endocrine evaluation includes a modern (post-2001) OECD 416 multigeneration study or OECD 443 Extended One-Generation Reproductive Toxicity Study (EGrRTS), and therefore will require new data generation for the majority of chemicals, since the dataset for many pesticides pre-dates the 2001 guidelines and must be supplemented. Hence the Guidance in effect sets new data requirements beyond existing EU legislation, and will result in an increase in animal testing. Some data can be provided by way of in vitro receptor binding assays, including ToxCast estrogen (but not androgen or thyroid) receptor data. The data most suited to endocrine evaluation are generated in mammals, but environmental effects in non-target species must also be assessed and a conclusion reached. Early feedback from some authorities is that mammalian data will not entirely reassure on potential environmental endocrine effects. There is a limited number of guidelines suitable for assessment of endocrine effects in environmental species, and Guidance recommends the OECD 240 Medaka EGrRTS and OECD 241 Larval Amphibian Growth and Development assays, with few laboratories currently offering these assays. It remains uncertain if thyroid hormone depletion will be concluded as endocrine disruption, or a secondary consequence. Experience to date indicates that considerable resources and time are required to conduct evaluations according to the Guidance particularly with respect to Appendix E, which then offers limited value to the process of assembling and integrating lines of evidence. Some regulators are requesting these evaluations with unrealistic short deadlines.

Oregon’s Approach to Systematically Select Toxicity Reference Values for a Program to Regulate Toxic Air Contaminants

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The State of Oregon recently adopted a risk-based approach to regulating toxic air contaminants from industrial sources. The approach, called Cleaner Air Oregon (CAO), requires source-specific risk assessments that consider exposures and associated risk to neighbors. With over 200 regulated toxic air contaminants, state agencies needed a streamlined method to select toxicity reference values (TRVs) for use in source-specific risk assessments. State agencies identified four federal and state program-specific authoritative sources of chronic toxicity information on toxic air contaminants: US Environmental Protection Agency’s (USEPA) Integrated Risk Information System, US EPA’s Provisional Peer Reviewed Toxicity Value program, the Agency for Toxic Substances and Disease Registry (ATSDR), and California’s Office of Environmental Health Hazard Assessment (OEHHA). For acute toxicity information, state agencies looked primarily to ATSDR and OEHHA. These agencies were identified as authoritative sources because they have the resources to conduct comprehensive literature reviews that consider the overall weight of scientific evidence, degree of consensus in the scientific community, and the quality of underlying studies when establishing toxicity information. In many cases multiple authoritative sources had toxicity information on the same toxic air contaminant. Initially, state agencies addressed this by establishing a hierarchy of authoritative sources, selecting toxicity information from sources higher in the hierarchy over other sources. In response to public comment, this approach was modified in the case of chronic TRVs such that the most recently published chronic toxicity information from among the authoritative sources was selected as the chronic TRV. State agencies continued to use a hierarchy of authoritative sources for selection of acute TRVs because some authoritative sources used averaging times that more closely matched CAO’s desired averaging period (24 hours) than others. The hierarchy of authoritative sources for acute TRV selection was ATSDR’s acute minimal risk levels (MRLs), OEHHA’s acute reference exposure levels, and ATSDR’s intermediate MRLs. As other states move towards risk-based regulation of toxic air contaminants, Oregon’s approach may serve as a helpful model.

Automated Data Visualization Tool to Map FDA-CDER’s Public-Private-Partnership Deliverables


One of the primary charges of the US Food and Drug Administration (US FDA) is to advance public health by streamlining drug development. Public-Private-Partnerships (PPPs) are collaborations between regulatory agencies and other stakeholders that make it possible to address areas of unmet need that are not the purview of any single organization. This project uses a tool developed from the D3JS (Data-Driven Document JavaScript) visualization library, to identify the activities and deliverables of the PPPs associated with US FDA’s Center for Drug Evaluation and Research (CDER), and assesses how they map to US FDA’s and CDER’s public health mission and congressional mandates. The aggregated PPP data are stored in a queriable PostgreSQL database and visualized via an interactive dashboard. The tool has been built with the goal of developing an automated platform for data visualization that can display organizational activities and their progress towards addressing specific public health needs.

Use of Dog Studies in US FDA’s Safety Assessments for Food Additives and Color Additives


The Center for Food Safety and Applied Nutrition (CFSAN) of the US FDA conducted a review to determine the impact of studies conducted in dogs on decisions regarding the safe use of food and color additives that have been the subject of petitions submitted to the Office of Food Additive Safety (OFAS). The Toxicological Principles for the Safety Assessment of Food Ingredients (Redbook 2000) provides guidelines for conducting toxicology studies in rodents (rats or mice) and dogs. We searched our database of food additive (FAPS) and color additive petitions (CAPs) with one or more dog studies and
identified 87 and 75 petitions, respectively. Dog studies were determined to be decisive in making a safety decision for 32% of FAPs and provided supportive information for 36% of FAPs. Dog studies were determined to be decisive in making a safety decision in 48% of CAPs and provided supportive information for 15% of CAPs. These preliminary findings indicate that dog studies have contributed to safety assessments of food and color additives; however, there has been a significant decline since the year 2000 in the number of dog studies submitted to US FDA for this purpose. Further analyses are aimed at identifying factors (e.g., study design, etc.) that influence the value of dog studies in regulatory decisions. US FDA is interested in identifying alternatives to the dog study that can be incorporated into safety testing strategies for food additives and color additives and that are consistent with US FDA’s goal of modernizing toxicology.

**2782 Pharmacology/Toxicology Review of Related Grades of Excipients in Generic Drug Products: Successful Practices and Common Pitfalls**

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US FDA’s Office of Generic Drugs (OGD) evaluates excipient safety to ensure that excipients in generic drugs maintain a similar safety profile to their respective reference listed drugs (RLDs). Sometimes the excipients used for pharmaceutical formulations may exist in various grades (i.e. subtle differences in their physical and or chemical characteristics). On occasion, generic drug applicants may submit a justification that relies on safety information from a related grade of an excipient that differs from the proposed grade in either physical or chemical characteristics. Such excipient grades, and their differences, require evaluation to determine whether their physical, chemical, and toxicological characteristics are relevant to support the safety of the proposed formulation. The authors surveyed 32 Abbreviated New Drug Application-Pharm/Tox reviews to identify trends in submissions, review considerations, successful practices and common pitfalls in excipient safety justifications. Of the reviews surveyed, data gaps were identified in approximately 33% of the submissions. These data gaps resulted in a non-approval and warranted additional information from the applicant. Data gaps consisted of incomplete reports, lack of relevant data to justify the safety of specific grade in question, or failure to address the drug’s context of use (i.e. local toxicity), safety of target population (e.g. pediatrics), duration of use (e.g. chronic). Additionally, applications received a deficiency if excipient grades could not be compared due to incomplete information on the physical, chemical and toxicologic characteristics of the grades. Successful safety justifications included similarities and differences in the physical and chemical characteristics across excipient grades, available safety information for both the proposed and the related excipient grades, and scientific justification for how the available data addresses the safety of the grade in question. Successful justifications also established the relevance of the available safety information to the context of use of the proposed generic drug product. Overall, this work highlights successful practices and common pitfalls in safety justifications to continually improve first-cycle generic drug approvals.

**2784 Better Ways Than the Bioassay: Weight-of-Evidence Approach to Assess Carcinogenicity Potential of Food-Use Pesticides**

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Rodent cancer bioassays have been used for decades to identify chemicals that may be carcinogenic to humans. However, numerous retrospective analyses and reviews of data collected from the bioassay over the past 40 years have raised questions about the relevance and regulatory need for the bioassay to assess risk to human health. As a result, a working group comprised of different sectors and stakeholders have developed case-examples using a weight-of-evidence (WoE) mechanistic-based approach to determine the appropriateness of waiving rodent cancer bioassays in the context of crop protection chemical registration. The WoE approach was used to evaluate exposure, mode-of-action, physiochemical properties, and toxicological data from defined endpoints. Selected pesticides that represented different classes of chemistry were reviewed using the WoE approach to evaluate available information on pesticide mode of action, indication for use, metabolic profile, toxicokinetics, genetic toxicology, histopathology from dose-response studies, tumour formation, hormonal perturbation, immune response, read-across, margins of exposure, uses, human exposure scenarios, and other relevant endpoints used in risk assessment. These data were collated to determine if there would have been sufficient information to perform a health protective chronic risk assessment without performing rodent cancer bioassays. Additional analyses are being conducted of a much larger set of pesticides to ensure adequate coverage of chemical space. The results of these analyses will be used to establish the criteria for when the mouse and/or rat cancer bioassay can be waived with sufficient confidence to protect public health.

**2785 California Law First in North America to Require Non-Animal Safety Substantiation of Cosmetic Ingredients**

E. Baker and K. Sullivan. Physicians Committee for Responsible Medicine, Washington, DC.

Growing public pressure on cosmetics companies is pushing the industry away from animal tests and towards greater uptake of in vitro and in silico methods. Although the US Food and Drug Administration does not require specific tests for cosmetics, many multi-use ingredients may be tested in vivo to meet regulatory requirements in other sectors or for non-required product stewardship reasons. Other products are subject to regulatory requirements in other countries. In 2018, California joined the European Union and other regions in severely restricting the sale of cosmetics products or ingredients that have been tested in animals. Starting January 1, 2020, no cosmetic may be sold within the state if it, or its ingredients, was tested on animals after January 1, 2020. Exemptions are allowed for testing conducted in response to a requirement by a state, federal or foreign regulatory body under certain conditions. Furthermore, the law requires that companies selling cosmetics or ingredients tested on animals under one of the exemptions use nonanimal
methods to substantiate the safety of the cosmetic or ingredient. Because the law applies to cosmetics companies as well as any third party suppliers or contractors, many ingredients manufacturers will now be required to use in vitro or in silico methods to substantiate the safety of their products, even if they are also conducting an in vivo study for the same endpoint to support the ingredient in another sector. Training and outreach efforts must be conducted to ensure ingredients suppliers are ready to comply with new regulations. Indeed, there are numerous non-animal methods available for assessing the potential hazards of cosmetics ingredients. This presentation will outline the legislative requirements and the available tools and resources to abide by them.

2786 PBT Assessment Criteria—Critical Tool or Policy Anachronism? An Analysis of Regulatory Approaches for Selection of Chemicals for Expedited Action

P. C. DeLeo¹, and S. Hartigan². Integral Consulting Inc. Annapolis, MD; and “American Chemistry Council, Washington, DC.

In June 2016, amendments designed to improve the Toxic Substance Control Act (TSCA), the primary chemicals management law in the United States, were codified into law. These included in new authorities in Section 6(h) that required the US Environmental Protection Agency (US EPA) to take expedited action on chemicals that are persistent, bioaccumulative and toxic (PBT). Unfortunately, the basis US EPA is initially using for identifying PBTs is a 2014 Agency prioritization document (the TSCA Work Plan) that did not rely upon rigorous collection or analysis of data. It also excluded from consideration any US EPA identified five chemicals for expedited risk management action by June 22, 2019: Decabromodiphenyl ethers (DecaDBE), Hexachlorobutadiene (HCBD), Pentachlorophenol (PCP), Phenol, isopropylated, phosphate (3:1; PIP 3:1), and 2,4,6-Tris-(tert-butyl) phenol (2,4,6-TTBP). An evaluation of the US EPA assessment of the use and exposure of the chemicals found that PBT criteria alone were not good measures of the need for expedited action. For example, no company had reported manufacture and/or import to the U.S. of two of the substance (HCBD and PCTP) in recent years, and nearly the entire market volume of another substance (2,4,6-TTBP) was consumed as chemical intermediate (methodology A) and a fuel antioxidant (6%). The evaluation of selection of chemicals for expedited action is particularly timely as US EPA is currently considering the next group of at least 20 chemicals which will be selected for risk evaluation, and US EPA has recently released a proposal for prioritization of the entire TSCA Inventory of active chemicals in commerce (approximately 30,000 chemicals). In light of the use and potential exposures for the TSCA Section 6(h) PBT chemicals, policy recommendations on approaches for selection of chemicals for risk evaluation and prioritization of the TSCA Inventory will be provided.

2787 The Nonclinical Innovation and Patient Safety Initiative (NIPSI): Supporting Human-Based Nonclinical Approaches through Advances in Regulation, Policy, Science, Education and Training

E. Baker. Physicians Committee for Responsible Medicine, Washington, DC.

The integration of modern and state-of-the-art nonclinical approaches for assessing drug safety, efficacy and disease modeling are expected to improve drug development. Recent advances, such as the US Food and Drug Administration’s (US FDA) Predictive Toxicology Roadmap and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States, represent a fundamental shift in how drugs will be developed and regulated. Regulators now state the need to move away from animal testing towards new approach methodologies that can be expected to be more predictive for humans, and have mapped their plans for doing so. The Nonclinical Innovation and Patient Safety Initiative (NIPSI) formed to foster stakeholder collaboration - among federal agencies, the private sector, and patient, health and research organizations - that supports innovative human-based science and addresses the factors that impede the uptake of modern, predictive approaches. Stakeholders initially met at a full-day NIPSI roundtable in January 2017 in Washington, D.C., then a growing group met during ancillary meetings of the Society of Toxicology Annual Meetings in March 2017 and March 2018. This presentation outlines NIPSI recommendations for advancing the uptake of human-relevant nonclinical approaches. One project aims to change US Food and Drug Administration (US FDA) regulations from requiring “animal” data to “nonclinical,” which encompasses animal in vivo and human and animal-based in vitro and in silico approaches. Another project involves lobbying the United States Congress to increase funding allocated for human-based science. The presentation will also include results from a recent review of NDAs for approved drugs that found no inclusion of “tissue chips” or “microphysiological systems” in US FDA submissions for approved drugs. Results will be updated for the Annual Meeting.

2788 Comparing Safe Intakes of Elements Established by the Food and Nutrition Board of the Institute of Medicine versus Those Established by the US Environmental Protection Agency

J. Ryer-Powder¹, and L. Ausman². ¹Environmental Health Decisions, Ladera Ranch, CA; and ²Tufts University, Boston, MA.

The Food and Nutrition Board of the Institute of Medicine (FNB) establishes Tolerable Upper Intake Levels (UL) for nutrients, defined as “the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to a given individual in the general population.” The US Environmental Protection Agency (US EPA) establishes oral reference doses (RfD) for chemicals in the environment, defined as “an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime”. The objectives of this study are to (1) compare ULs with RfDs for elements, (2) examine the basis for the levels, and (3) determine if results support establishing a uniform approach to setting safe levels for elements in food and the environment. Current ULs and RfDs for male adults were obtained from the National Institutes of Health (NIH) toxicology database and US EPA website. Tolerable ULs were compared with RfDs and a ratio was developed to calculate differences in these values. For each element, selection of (1) the studies relied upon, (2) the critical adverse health effect, (3) the no observable adverse effect level (NOAEL) or lowest observable adverse effect level (LOAEL), and (4) uncertainty factor (UF) for RfD were evaluated to assess differences in levels. The difference in UL/RfD ratios ranged from 0.7 to 5.7. The largest differences were seen with copper, fluoride, molybdenum, and vanadium. The bases for the differences were selection of studies, critical adverse health effects, selection NOAELs or LOAELs, and UF. The FNB and US EPA rely on similar methodologies to set “safe” levels for intake of elements. Both procedures select relevant studies, derive a NOAEL or LOAEL, and divide the level by an UF to account for uncertainties (e.g., extrapolation from animals to humans and susceptibility to adverse effects). Although the intent of ULs and RfDs is to use the weight of the evidence to define a safe upper limit of exposure, different results were obtained for certain elements. It may be prudent for agencies to harmonize the setting of safe levels of nutrients for exposure via the diet and environment.

2789 A Case Study on Parabens Shows the Applicability of New Generation Risk Assessment Based on New Approach Methodologies

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The Long Range Science Strategy (LRSS) is Cosmetics Europe (CE) research programme on new generation risk assessments (NGRA) based on new approach methodologies (NAMs). It includes a series of case studies, and one of them is on parabens. We use a tiered workflow, coming from the formed Seurat-1 programme and published by the OECD. The goal is to perform a safety assessment of parabens with long or branched lateral chain based on existing information from the parabens that have short lateral chain and for which toxico-kinetics and toxico-dynamic information are available, in combination to aggregate exposure evaluation. At Tier 0 we collected information for agencies to harmonize the setting of safe levels for exposure via the diet and environment.

¹, ², and ³, and L. Ausman². ¹Environmental Health Decisions, Ladera Ranch, CA; and ²Tufts University, Boston, MA.

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action and toxicodynamic (e.g., docking studies, ToxCast® data, endocrine-receptor assays, transcriptomics). The conclusion on the safety, here, is based on read across using both chemical and biological similarities.

### 2791 Characterizing In Vitro Tests Co-Culture for Safety Assessment: Characterization of Testes Longitudinal Cellular Dynamics In Vitro

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To improve chemical evaluation and identification of potential adverse health effects in humans, we are designing in vitro methods. In vitro cell cultures derived from a single cell type are limited. Using a 3 dimensional organotypic approach allows us to evaluate systematic interactions between cells and reduces the need for in vivo assessments. This organotypic, in vitro model of testicular development mirrors the development shown by in vivo studies. Isolated testicular tissue was harvested from C57BL/6 male mice on post-natal day 9 and digested into a single cell suspension and co-cultures were maintained for up to 16 days in 24 well plates with data collection on days in vitro (DIV) 3, 7, and 16. These times represent “windows of potential susceptibility” for testes development. Markers for Sertoli cells (Vimentin), Leydig cells (3β-HSD), Germ cells (DAZL, SCP3 and c-kit), and proliferation (PCNA) were quantified. For each well, we used an immunofluorescence dual staining technique with Scanning Laser Image Cytometry (iCytoS) to observe the percentage of cell type changes among the population and distribution of each cell population. Our observations in vitro demonstrated concordance of changes in Sertoli cells, Leydig cells and Germ cells throughout testicular development with similar trends in vivo. This project is supported by US EPA (RD 83573801, RD 83451401), the NIEHS (SP01ES009601, SP03ES00703, T32ES015459), and US FDA (1U01FD004242). The views expressed in this paper are those of the authors and do not necessarily reflect the views of the US EPA.

### 2792 Tiered Approach to Chemical Screening Using Both Hazard- and Exposure-Based Evaluation

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With growing interest in the chemistry of everyday products, there is a greater need for methods of product safety assessment. There are a number of alternatives assessment frameworks that screen products and ingredients using scoring methodologies based on a chemical’s inherent hazard. However, risk is a function of both hazard and exposure. Potential for exposure is often dependent on whether the chemical is released from a product and the level of individual exposure. Comprehensive risk assessments that consider both hazard and exposure generally require substantial data input as well as quantitative models that may be time consuming to apply. Thus, there is a need for streamlined alternatives methods that allow efficient screening of products to prioritize those calling for comprehensive risk assessment versus those where exposure levels and hazards are expected to be of low concern. ACC’s Sustainability and Market Outreach Division has developed a tiered approach to chemical screening that provides a simple, yet science-based method for evaluating the potential for chemical exposure from a product. This more holistic framework for alternatives assessment can be used to introduce the concept of risk and exposure into existing hazard-only methodologies. The approach begins with an initial identification of relevant exposure scenarios and pathways based on product use, as well as identification of product components. The first tier of the approach screens ingredients using basic information on chemical hazard. The second tier screens ingredients based on an understanding of how product form and ingredient chemical and physical characteristics influence exposures. The third tier provides a risk scoring methodology that combines information on the intended use of the product and additional information on hazard. The final output is a risk matrix that users can employ to prioritize their actions and resources towards the highest risk scenarios. ACC performed a pilot test of 10 generic, unbranded formulations to identify the benefits and challenges of the approach, and potential opportunities for improvement or refinement. ACC is currently working to implement recommendations developed as a result of the pilot test.

### 2793 Active Machine Learning for Information Retrieval in Systematic Literature Reviews: Addressing Bias and Appropriate Use


Systematic reviews in toxicology and human health risk assessments involve the retrieval and structured examination of information from all relevant sources related to a research question of interest. Systematic reviews commence with a broad-based literature search that is designed to be comprehensive and therefore tends to return a high proportion of non-relevant articles. Text analytics and natural language processing technologies are now widely used to save time and increase efficiency in the information retrieval and prioritization steps of a systematic review by automatically discarding non-relevant articles. For example, supervised machine learning approaches rely on a training dataset (a set of documents labelled, for instance, as relevant or not to a topic of interest) to build models that go on to automatically classify a larger set of unclassified documents. Recently, “active” machine learning was proposed as a solution that limits the cost of creating a training dataset by sequentially focusing on training only the most informative documents. We simulate active machine learning by using a set of approximately 7,000 abstracts from the scientific literature related to the chemical arsenic that was previously classified by subject matter experts with regard to relevance to mode of action. We examine the performance of alternative sampling approaches to sequentially expanding the training dataset, specifically looking at uncertainty-based sampling and probability-based sampling. We discover that while such active learning approaches can potentially reduce training dataset size by up to 60% compared to random sampling (regular supervised machine learning), active machine learning-based predictions of model performance potentially suffer from bias that negates its potential benefits. Without correction, we find that active sampling can result in biased performance metrics up to a level of 25 percentage points. We discuss statistical approaches and the extent to which the bias resulting from skewed sampling can be compensated. We find that the use of independent validation methods can help reduce but not eliminate bias in active learning. We propose a useful role for active learning in contexts where accuracy metrics are not critical and/or where it is necessary to rapidly retrieve a subset of relevant literature.

### 2794 Using Interactive Media to Explore and Explain Hazard Assessment Data

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Technological and methodological advancements in systematic review allow us to gather, screen, and characterize large literature bases supporting hazard assessment, risk assessment, and risk management at an increasing rate. Properly assessing and interpreting these data and presenting them to the public in a transparent manner becomes increasingly important as the risk assessment process faces increased scrutiny from scientific, political, and public communities. This is particularly important for hazard assessment, when health and human lives may be dependent on data interpretation. Data visualizations can address these areas of need by helping viewers perceive, understand, and assess the displayed information easily and quickly. Recently, more government agencies are leveraging new kinds of data visualizations in public reports, some of them web-based and interactive. In this analysis we explored how online systematic review tools such as HAWC and DRAGON ONLINE provide methods to rapidly capture trends and findings in a literature base with the aim of identifying best practices for implementing visual and interactive media. We also used visualization software and packages such as Tableau and R Shiny, which facilitate creation of interactive dashboards that viewers can manipulate to ‘zoom in’ on specific parameter values, to create ‘Evidence Maps’ for scooping available literature and dose-response, hazard, margin-of-exposure, and uncertainty range data. We present recommendations for the use and display of these maps, including key features and examples, and lessons learned from using them in risk assessment. We will also discuss how incorporating interactivity into visualizations is a highly effective method of targeting visualizations for use in two contexts: 1) Exploring data - allowing risk assessors to examine complicated datasets for scoping and analysis, and 2) Explaining data - increasing the transparency and clarity with which data and analysis are presented to risk managers and the public.
There is increasing need to rapidly determine chemical hazards in the DOD. However, questions surround the application of new approach methodologies (NAM) for rapid hazard assessment. To address this, DOD is developing a strategy for using NAMs to rapidly and accurately assess chemical hazards. We identified six key areas to address in develop in a roadmap: 1. DOD needs are different from other Federal agencies; DOD needs to understand hazards of new materials, chemical weapons, chemical spills, balance use with health hazards, and under diverse conditions (climate, urban, suburban, high altitudes). 2. Barriers to using NAMs to be addressed: Lack of acceptance by US EPA or OECD, unfamiliarity, hesitancy to work with developers of NAMs, no DOD service policy supporting use during acquisition process. 3. Increase technical acceptance of NAMs: verify and validate NAM performance, collaborate with others that promote regulatory acceptance, demonstrate NAMs in case studies, define technical and assay/model uncertainty in predicting human or standard model endpoints, explain limitations/relevance, use of NAMs is not an all or nothing strategy (2R1 + 1R). 4. Identify technology/innovation gaps and solutions: Gaps include inter-individual and laboratory variability, dose-response extrapolations, systems toxicology approaches, extrapolation to human relevance, few ecotoxicology NAMs and understanding mixture effects. Solutions include: new in silico techniques, leveraging Federal efforts, collaborate with non-DOD facilities, archived tissues to develop endpoints for analysis (e.g. `omics), foster tissue on chip/synthetic systems. 5. Education and outreach will be required support use of NAMs including better coordination of efforts across DOD and Federal agencies. 6. Develop an implementation plan and identify policy arising from roadmap implementation. A draft road map has been authored addressing these issues and is undergoing further review by DOD and other Federal partners. We expect that the roadmap will accelerate the ability of DOD services to understand chemical health hazards, protect DOD personnel and respond to emergency chemical situations.

Toxicological Review of Inorganic Arsenic

The Integrated Risk Information System (IRIS) Program is developing an updated Toxicological Review of Inorganic Arsenic. Given the size and complexity of the existing reference database, the National Research Council recommended US EPA conduct analyses to further prioritize among its three tiers of health outcomes. To prioritize outcomes for dose-response, US EPA conducted an analysis that considered strength of evidence of the epidemiological evidence for hazard (either by relying on conclusions from other assessments or by conducting new systematic reviews of the literature). Human studies containing exposure- or dose-response data were evaluated to assess aspects of internal validity (risk of bias) of study findings based on study design and conduct for hazard identification using an approach adapted from the Office of Health Assessment and Translation (OHAT). Identified strengths and limitations were considered to reach a study confidence classification of High, Medium, or Low confidence, or Uninformative for a specific health outcome. Judgments regarding the strength of the human evidence were made considering risk of bias, study sensitivity, consistency, strength, biological gradient, and coherence. Datasets from outcomes judged to have a "robust" or "moderate" evidence base were further evaluated for their suitability for dose-response analysis and included in a margin of exposure (MOE) screening level dose-response. More than 250 separate datasets were modeled using US EPA's Benchmark Dose Software. Points of departure based on the maximum likelihood estimate of the dose that increased the relative risk by 20% (RRD) were compared with an estimate of the general US population exposure to inorganic arsenic to derive MOE values for each dataset. The utility of dose-response analyses for economic benefit-cost analysis was also considered. MOE analysis results were used to inform the selection of health outcomes for further dose-response analysis. Animal and mechanistic studies are considered supplementary. Based on the strength of evidence evaluation and MOE analysis the arsenic assessment focuses on the following six health outcomes (out of fifteen): bladder cancer, lung cancer, diseases of the circulatory system, pregnancy outcomes, neurocognitive effects, and diabetes. The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the US EPA.
in NHANES and the effect levels reported in the human health effects literature. Our conclusions show the machine learning techniques that we used were found to be comparable to expert review of literature and increased efficiency, and we present preliminary results from our investigations of 5 environmental chemicals as an example of how this type of screening-level approach may qualitatively inform public health considerations and cumulative risk decisions. The duration of the exposure is contingent on product use, life-cycle, and exposure control decisions.

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**2800 Detection of Public Health Risk Levels for Contaminants in Consumer Products**

B. J. Hughes, V. S. Bhat, and K. D. Cox. NSF International, Ann Arbor, MI.

NSF, as a third party certifying body, determines when very high levels of chemicals extracted from certified products present a significant public health that may warrant immediate risk management measures (e.g. product holds or recalls). Existing paradigms lack sufficient chemical- and product-specific exposure context. Chemical identification/characterization, product life-cycle, population(s) affected, mechanistic and toxicological data need to be considered when deriving health-protective and scenario-specific guidelines. This phase of the pipeline identified 97% of duplicate groups with a precision of 52%. After accounting for manual review to remove false positives from the machine-identified duplicates pile, the combined pipeline realized a 99% efficiency gain. We normalized these results based on maximum possible efficiency gains (which depends on the proportion of duplicate groups in the original dataset) to estimate a normalized efficiency gain of 94%. Finally, we utilize text analytics to analyze the distribution of keywords across a combined set of references to inform refinements in search strategies. We show how iteratively streamlining search keywords based on their retrieval characteristics could reduce manual labor by an additional 15% downstream in the systematic review process.

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**2801 Comparison of NTP OHAT and US EPA TSCA Study Quality Criteria: Trichloroethylene (TCE) and Congenital Heart Defects (CHDs) as a Case Study**


As part of developing best practices in systematic review (SR) for toxicity and risk assessment, tools are being designed or refined to accommodate evidence bases which include many different types of studies (i.e., experimental, in vitro, and observational studies). This is necessary as such tools were originally designed for different evidence bases (i.e., clinical trials). In this case study, two tools available to appraise data quality are compared as means of providing an understanding of the practical application of available approaches. Using the evidence base for TCE and CHDs, data were appraised using two tools: (1) the US EPA TSCA draft guidance for study quality evaluation SR principles, and (2) the NTP-OHAT Risk of Bias tool. Nine observational studies, 10 experimental animal studies, and 20 mechanistic studies were assessed representing a diverse range of study designs (in vivo, in vitro, and in vivo), durations, and exposure routes. The application of TSCA metrics to the TCE-CHD literature demonstrated some similarities with the NTP-OHAT criteria [e.g., none of the human studies were considered high quality (TSCA score <1.7 or OHAT Tier 1), and within the experimental animal studies, the inhalation animal studies were determined to be of higher quality than the oral studies]. However, there were more differences than similarities. Most notably, strict adherence to the TSCA metric criteria definitions resulted in designations of many human and mechanistic studies as “unacceptable” (and subsequently not considered in the risk assessment). A key difference between the tools and resulting quality characterizations is how the absence of reporting a metric is appraised. This was particularly impactful for several mechanistic studies, as several study design elements (e.g., cell plating and maintenance, cytotoxicity, etc.) were not often reported. Additional differences can be attributed to the thoroughness and specificity of the TSCA approach, as well as breadth of the TSCA criteria (includes multiple aspects of study validity), relative to NTP-OHAT. This case study demonstrates the need for continued refinement of critical appraisal tools to assess study quality.
There are some criticisms of the US and EU approaches about the environmental (Risk) Assessment (E(R)A) of medicinal products. They only consider one a particular medical product at a time, and ignore the fact that some compounds can cause additive or even synergistic toxic effects when in the presence of other APIs with similar Mechanism of Action (MoA). Recent evidence strongly supports the fact that amongst the pharmaceuticals in use today, steroidal pharmaceuticals merit particular attention for their adverse effects on the environmental compartment, i.e. glucocorticosteroids (GC). Synthetic GCs have been designed to have higher glucocorticoid potency, reduced mineralocorticoid effects and a longer duration of action. Synthetic GCs share the same biochemical backbone as endogenous cortisol, but in most of the tissue-specific biodynamics, H in the 9th carbon is substituted by F or CI to increase the stability of these compounds in the human body. Therefore, it is anticipated that the corticosteroids with F or CI substitution could resist degradation in sewage treatment plant (STP). Experimental data show that GCs disrupt metamorphosis in anuran amphibians by complex modes of action and suppress reproductive functions in fish as an adaptive response to divert metabolic building blocks away from biosynthetic pathways. Moreover, in fish GCs cause follicular atresia, advance or delay oocyte maturation and ovulation or affect egg size, fertilization success. GCs are classifiable as endocrine disruptors. Interestingly, under EU law if the medicine has endocrine disrupting properties the applicant is required to perform an E(R)A. Conversely, US law does not consider the risk posed by endocrine disrupters to aquatic species. It is very likely that pharmaceuticals with similar MoA will act in an additive manner, and therefore it can be argued that the total concentration of GCs in the environment, rather than the concentration of each individual GC, is of most relevance to the risk assessment of GCs on aquatic organisms. Therefore, in order to overcome the cocktail effects, we recommend that E(R) A is performed for groups of APIs with similar MoA. This would enable a more accurate E(R)A and a reduction of test organisms. The group approach for E(R) A can lead to a reduction of costs for all the companies marketing APIs with similar MoA.

Guidelines for determining a safe, oral dose for a compound in humans using laboratory animal test data differ based on the source of exposure (e.g., drug or therapeutic, drinking water contaminant, food substance, or dietary ingredient). A lack of harmonization between methodologies introduces confusion and variations in the application of regulatory guidance and determinations of safe, oral doses in humans. For orally administered drugs, US FDA recommends converting any observable adverse effect levels (NOAELs) established in lab animals to NOAEL human equivalent doses (HEDs) using body surface area conversion factors (BSA-CFs) to normalize for differences in body surface area between the test animal and humans. An uncertainty factor (UF) (e.g., default of 10) is then applied to the NOAEL_HED to derive Maximum Recommended Starting Doses (MRSDs) in clinical trials. Similarly, US EPA guidance recommends calculating oral reference doses (RFDs) by applying the appropriate composite UF to a NOAEL_HED. The US EPA converts a NOAEL in lab animals to a NOAEL_HED by physiologically based-toxicokinetic (PBTK) modeling or chemical-specific information. Absent such data, dosimetric adjustments are made to the RFDs. However, when considering exposure to multiple substances, the PDE values obtained in 5 groups (being differentiated by the selection of preferred values) was summarized in a memorandum generated by members of US EPA’s Office of Land and Emergency Management Human Health Regional Risk Assessors Forum Toxicity Workgroup (and with support from the Superfund Technical Support Center). After discussion and resolution of the toxicological issues for these chemicals by the Toxicity Workgroup, changes will be proposed to management for inclusion into the RSLs and baseline risk assessments.

The “Regional Screening Levels for Chemical Contaminants at Superfund Sites” are calculated from human health toxicity values and national default exposure parameters, and used by all US EPA regions to aid in decision-making at Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) sites. Human health toxicity values in the RSLs are selected based on a recommended hierarchy of sources, namely 1) US EPA’s Integrated Risk Information System (IRIS); 2) Provisional Peer-Reviewed (PPRTVs); and 3) other (“Tier 3”) values. However, the hierarchy provides flexibility to encourage risk assessors to select values according to the “best science” available. In some cases (for 11 inhalation toxicity values and 21 oral toxicity values), a subchronic toxicity value from the hierarchy of sources is lower than its corresponding chronic toxicity value. To evaluate and select a definable toxicity value for inclusion in the RSLs and baseline risk assessment, information was compiled that includes the source and the basis of the toxicity value, the critical effect(s), the point of departure (POD), the composition uncertainty factor (UF), and the resultant toxicity value. Differences among subchronic and chronic toxicity values (for the same chemical) were attributed to changes in: 1) the selection of a principal study (e.g., newer assessments based on a more recent publication); 2) methodology (e.g., the use of benchmark dose (BMD) modeling instead of a NOAEL/LOAEL approach, and the application of uncertainty factors or dosimetric adjustments); or 3) a combination of these factors. Based on evaluation of the data, preferred toxicity values were selected for each chemical. The values and rationale for the selection of preferred values was summarized in a memorandum generated by members of US EPA’s Office of Land and Emergency Management Human Health Regional Risk Assessors Forum Toxicity Workgroup (and with support from the Technical Support Center). After discussion and resolution of the toxicological issues for these chemicals by the Toxicity Workgroup, changes will be proposed to management for inclusion into the RSLs and baseline risk assessments.

Guidelines for determining a safe, oral dose for a compound in humans using laboratory animal test data differ based on the source of exposure (e.g., drug or therapeutic, drinking water contaminant, food substance, or dietary ingredient). A lack of harmonization between methodologies introduces confusion and variations in the application of regulatory guidance and determinations of safe, oral doses in humans. For orally administered drugs, US FDA recommends converting any observable adverse effect levels (NOAELs) established in lab animals to NOAEL human equivalent doses (HEDs) using body surface area conversion factors (BSA-CFs) to normalize for differences in body surface area between the test animal and humans. An uncertainty factor (UF) (e.g., default of 10) is then applied to the NOAEL_HED to derive Maximum Recommended Starting Doses (MRSDs) in clinical trials. Similarly, US EPA guidance recommends calculating oral reference doses (RFDs) by applying the appropriate composite UF to a NOAEL_HED. The US EPA converts a NOAEL in lab animals to a NOAEL_HED by physiologically based-toxicokinetic (PBTK) modeling or chemical-specific information. Absent such data, dosimetric adjustment factors (DAFs), based on body weight scaling, are applied to the NOAEL in animals to calculate the NOAEL_HED. In contrast, HEDs are not included in US FDA regulations or guidance for food substances or dietary ingredients. When assessing the safe use of a food substance under the conditions of intended use (i.e., Generally Recognized as Safe (GRAS) dossier or Food or Color Additive Petition), NOAELs are identified in lab animals, then the NOAEL from the most sensitive laboratory species is commonly selected, and an UF (e.g., default of 100) is applied to the NOAEL to determine the Acceptable Daily Intake (ADI) in humans. It is common for the NOAEL in a lab animal species to be extrapolated to humans based on body weight alone (1:1), to determine the margin of safety (MOS) between the (extrapolated) NOAEL in humans and estimated daily intake (EDI). For dietary supplement ingredients, an ADI may also be determined through the identification of a NOAEL in lab animals and application of a composite UF, without consideration for allometric scaling. These differences afford the opportunity for inconsistencies and variations in translating safe, oral doses, highlighting the need for additional education within the scientific community, and consensus and harmonized guidelines between US regulatory bodies.

The EMA/CHMP/SWP/10430/2012 document introduced a new criteria to define the limits of exposure to establish the risk in multiproduct GMP pharmaceutical facilities and to manage the possible cross contamination issues. This involves the determination of exposure limits according to toxicological criteria based on the inherent characteristics of each substance (substance-based approach). The procedure followed for determination of health based exposure limits for substances for a Technical Support Center (TSC). After discussion and resolution of the toxicological issues for these chemicals by the Toxicity Workgroup, changes will be proposed to management for inclusion into the RSLs and baseline risk assessments.
Systematic review approaches in human health assessment includes the integration of evidence from experimental, epidemiological, and mechanistic studies. The process of screening and evaluating evidence can be challenging due to the diversity of research models, methods, outcomes, and the variety of known mechanistic pathways resulting in chemical-induced toxicity. The Ten Key Characteristics of Carcinogens provide a useful method for searching and screening mechanistic information on chemical-induced carcinogenesis. The goal of the current project was to identify a set of key characteristics that can be used to analyze mechanistic evidence which may inform the potential pathways/networks associated with chemical-induced male reproductive toxicity. The proposed key characteristics were identified through a review of established mechanisms for chemical-induced male reproductive toxicity. An expert workshop was convened in March 2018 at the University of California-Berkeley to determine whether the key characteristics approach can also be applied to non-cancer effects including male reproductive toxicity. Eight key characteristics were identified and include alterations in: 1. germ cell functions, 2. somatic cell functions, 3. reproductive hormone levels/production, 4. hormone receptors, 5. DNA damage, 6. epigenetic modifications, 7. oxidative stress, and 8. inflammation. As a proof of principle, the key characteristics were used to evaluate evidence from mechanistic studies on the PCB mixture Aroclor 1254. A database was developed to capture experimental design information and mechanistic outcomes from the available studies. The resulting database can be used to qualitatively evaluate mechanistic and toxicological evidence as part of a hazard characterization analysis. Disclaimer: The views expressed are those of the authors and do not necessarily represent the views or policies of the US EPA.
Nephrotoxicity is among the top five reasons for clinical attrition of compounds. Current in vitro models for detecting and de-risking nephrotoxicity are often inadequate as they lack apical and basolateral transporters responsible for compound uptake and disposition. Additionally, animal models suffer from poor predictivity for their human counterparts. Thus, better and more predictive models are needed which can be used for safety assessment of nephrotoxic compounds. Herein, we evaluated the utility of freshly isolated human primary proximal tubule cells (aProximate™) seeded on TransWell plates for de-risking nephrotoxicity using kidneys from multiple different donors. Freshly isolated proximal tubule cells retained many of the key transport and metabolism functions that are both critical for toxicity and lost during cryopreservation. To assess the utility of aProximate cells, a variety of 30 mechanistically distinct pharmaceuticals were screened using translational safety biomarkers such as KIM-1, NGAL, and Clusterin to detect toxicity in vitro in addition to non-specific end points such as ATP depletion, LDH leakage, and TEER. NGAL showed the highest predictivity with a specificity of 85%, specificity of 75% and area under the ROC curve of 0.86. Importantly, the model was able to correctly rank-order compounds from the same chemical class according to their clinical risk of causing drug-induced kidney injury. Using the cut-offs generated by the 30-compound dataset, an additional 10 Takeda internal compounds were screened, and the assay could distinguish nephrotoxic compounds from benign thus validating the predictivity of the current platform. This in vitro model shows potential as a robust platform for safety assessment of nephrotoxic compounds.

2811 Freshly Isolated Primary Proximal Tubule Cells as an In Vitro Platform for Nephrotoxicity Testing

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2812 Lead (Pb2+)-Induced Increases in Calcium Oxalate Crystal Formation Is Ameliorated via IP3-Receptor Knockdown

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Calcium oxalate (CaOx) accounts for 75-80% of kidney stone composition with a high recurrence rate. Genetic predisposition, gender, geographic region, diet, and low fluid intake all contribute to disease pathogenesis. However an important contributor to CaOx crystal formation that remains insufficiently studied is chronic exposure to environmental pollutants, specifically nephrotoxic metals. Lead (Pb2+) is of particular interest as epidemiological data indicate that low-level, chronic exposure (BL = 0.48-3.85µM) confers a 35% increased risk of developing CaOx nephrolithiasis. However, the mechanisms underlying association between kidney stone formation and lead exposure have yet to be elucidated. Interestingly, Pb2+-induced increases in inositol 1,4,5-trisphosphate (IP3) with subsequent IP3 receptor (IP3R) activation mobilizes free Ca2+ in rat proximal tubule cells. Drosophila provide a useful genetic model where major renal pathophysiology, particularly specific nephrotoxic-mediated pathways, can be efficiently studied. Anterior Malpighian tubules (MT) were isolated from either w1118 or IP3R knockdown female flies (n=13-15) and treated with oxalate (5mM) ± Pb2+ (2µM) for 1h. Following exposure, MTs were imaged using differential interference microscopy (DIC), and crystals quantified using ImageJ. CaOx crystal number and total area were significantly increased (~5-fold) in Pb2+-treated MTs compared to oxalate-exposed MTs when compared to oxalate alone controls. However, CaOx crystal number and total area in Pb2+-treated MTs were significantly decreased (~3-fold) indicating a role for IP3R-mediated Ca2+ mobilization as a mechanism for Pb2+-induced increases in CaOx crystallization. Future studies will determine whether IP3R transgenic flies reared on a Pb2+-containing diet mimic these in vivo results.

2813 Diglycolic Acid, the Toxic Metabolite of Diethylene Glycol, Affects Calcium Homeostasis, Mitochondrial Respiration, and the Aspartate–Glutamate Carrier Citrin in Human Proximal Tubule Cells

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Diethylene glycol (DEG) is an organic compound that is found in household products, including brake fluid, but can also be an adulterant in medicines by acting as counterfeit glycerin. DEG poisonings affect the liver and nervous system, but have been characterized predominately by acute kidney injury and specifically necrosis of the proximal tubule cells. Diethylene glycol is metabolized to two primary metabolites: 2-hydroxyethyoxycetic acid (2-HEAA) and diglycolic acid (DGA). In human proximal tubule (HPT) cells, DGA is 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. In other studies using cells with sub-threshold concentrations of DGA were conducted using primary human proximal tubule cells. Cells treated with DGA and BAPTA-AM, a calcium-specific chelator, were harvested and analyzed using the ratiometric calcium indicator dye Fura 2-AM. Additionally, HPT cells were treated in a DGA time-course with either additional calcium or a calcium ionophore to see if the effects of DGA on oxalate transporter phosphorylation could be attenuated. This oxygen consumption rate was measured using the Seahorse XF24 Analyzer. Citrin expression in DGA-treated HPT cells over time was also measured via western blot analysis. DGA treatments showed an increase in intracellular calcium at 25 mM at 36 and 48 h, but the BAPTA-AM-treated cells at varying concentrations were inconclusive. DGA decreased OCR in a time-dependent manner, lending support to the effects of DGA on energy production. Citrin expression was decreased with DGA incubations compared to control at the 36 and 48 h timepoint. These data suggest that DGA increases total HPT intracellular calcium levels, but more studies using other calcium sensing techniques need to be performed to determine the location and other targets of this effect.

2814 Efflux Pathways of Diglycolic Acid in Primary Human Proximal Tubule Cells

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Diethylene glycol (DEG) is an industrial solvent, generally found in brake fluid and lubricants, implicated in mass poisonings worldwide. The predominant metabolites of DEG are 2-hydroxyethyoxycetic acid (2-HEAA) and diglycolic acid (DGA). Exposure to DEG causes proximal tubular necrosis, as well as hepatoxicity and a delayed peripheral neuropathy. DGA has been implicated with the metabolite responsible for the renal and hepatic toxicity seen in these poisonings, as it is shown to accumulate in the organs. Due to this accumulation, transport of DGA into and out of kidney cells is of interest. Initial experiments focused on both the organic anion transporter (OATs) and the sodium-dependent dicarboxylate transporters (NADCs). Results from these experiments showed that stimulation of OATs did not enhance DGA efflux. However, primary human proximal tubule (HPT) cells have been reported to lose OATs activity over time in culture. Therefore, it was important to determine if OATs were functioning in our cell model. We hypothesized that preloading the cells with dicarboxylate substrates of OAT1 and OAT3 would cause an increase in uptake of the typical OAT substrate para-aminom hippurate (PAH). HPT cells were grown on inserts until confluent, then loaded apically with succinate, DGA, glutarate, α-ketoglutarate, or control buffer. After incubations, cell lysates were counted on a scintillation counter to determine PAH uptake. Loading with succinate was able to increase the PAH uptake, while DGA was not able to increase PAH uptake. We concluded that OAT1/3 were functional in our cell model and that DGA was not likely removed from the cells for exchange for PAH.

2815 Development of Human Proximal Tubule-Chip for Assessment of Potential Renal Transporter-Based Drug-Drug Interactions


The kidney plays a key role in elimination of xenobiotics and endogenous compounds through its complicated and efficient uptake and efflux transport systems. An investigation of drug interactions with renal transporters and in upregulating drug disposition and toxicity, and more importantly, predict potential drug-drug interactions in human. However, currently available cell-based models often failed to predict renal transporter activity and not scalable to a predictive clinical outcome due to in vitro in vivo discrepancy. Our aim was to develop a human Proximal Tubule-Chip for assessment of renal transporter-based drug-drug interactions. Our Proximal Tubule-Chip is a microengineered microphysiological system where human proximal tubule cells and glomerular microvascular endothelial cells were cultured under continuous medium flow and mechanical forces. Human primary proximal
tubular epithelial cells were cultured in the tubular channel on top of the porous membrane whereas glomerular microvascular endothelial cells were cultured on the opposite side of the same membrane in the vascular channel under continuous physiological flow to form functional Proximal Tubule-Chip. The Proximal Tubule-Chip maintained polarized proximal tubule cell morphology for up to 15 days, exhibited tight junctions, barrier function (inulin leakage), demonstration of proximal tubule cell marker proteins including aquaporin-1 and Na/K-ATPase, revealed gene expression by real-time quantitative polymerase chain reaction of renal transporters including glycoprotein, multidrug and toxin excision (MATE) 1, MATE2-K, organic anion transporter (OAT) 1 and 3, and organic cation transporter (OCT2). We also demonstrated that the functional activities of proximal tubule cells, para-aminomuconic acid mediated by OAT1/3 and creatinine mediated by OCT2. These results suggest that the Proximal Tubule-Chip represents a physiologically relevant system for drug discovery and development applications.

2816 Supplementation of Growth Media of Cultured Renal Cells with the Energy Substrate Acetoacetate Impacts Energy Metabolism and Response to Toxins

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The proximal tubule of the kidneys is a metabolically active tissue with a high energy demand which makes it susceptible to factors that interfere with oxidative metabolism. Cultured kidney cells such as LLC-PK1 possess many functions of the proximal tubule, however their energy metabolism differs from the highly oxidative tubule metabolism in vivo. This may impact its reliability in predicting toxicity. Modulating the growth media composition has been shown to shift cultured liver and muscle cells from high glycolytic activity to increased oxidative metabolism and enhanced ATP production, but little is done on renal cells. This study is designed to examine the influence of culture media composition on the energy metabolism of LLC-PK1 cells and their responses to mitochondrial toxins. Cells were grown to confluence in SFDM media containing 3% FBS and 5 mM glucose or 5 mM glutamate supplemented with 10 mM acetoacetate (0.5) increased for cells grown with acetoacetate (30.6 ± 4.11 pmol O2/min/μg DNA compared to 20.9 ± 2.22 for 5 mM glucose alone). Other measures of mitochondrial function were also significantly increased for cells grown with acetoacetate including basal respiration, maximal respiration, ATP-linked respiration, and coupling efficiency. No significant changes in measures of glycolysis were detected between the two treatments. Two compounds which have been shown to affect mitochondrial function were examined to determine whether the culture media would influence their toxicity. Cells treated with the antifungal clotrimazole for 24 hr exhibited significantly lower LC50 (17.3 ± 3.7 μM) compared to cells grown in glucose alone (34.1 ± 3.9 μM). In contrast, cells treated with the NSAID diclofenac for 24 hr showed no effect of media composition. These results indicate that the composition of the growth medium influences the energy metabolism of cultured renal cells which may affect the responses of the cells to toxicants.

2818 Development of Predictive In Vitro Cross-Species Proximal Tubule Models for Drug Transporter and Nephrotoxicity Studies

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Currently there is no in vitro platform that enables cross-species comparisons of renal drug transport or nephrotoxicity, which is one of the reasons for the high incidences of drug attrition. Our solution to this is to develop and characterise novel assay platforms using primary renal proximal tubule cells (PTCs) derived from key model species. Here we report the extension of the aProximate™ assay platform to include non-human primate (NHP) and canine PTC models. PTCs were isolated from fresh kidney cortex using a collagenase/enzymedigested protocol. NHP and canine PTCs grown on Transwells formed confluent monolayers and produced average TEER value of more than 90 Ω.cm² after 7 days in culture, similar to human and rat PTC monolayers. At the mRNA level, NHP and canine PTCs expressed key drug transporters including MDR1, MRP4, BCRP, OCT2, OATP4C1 and lower levels of OAT1. Functionally, a net digoxin secretion across NHP monolayers; 10μM digoxin, the apical to basolateral flux (J+ apical to basolateral flux (J+ apical to basolateral flux (J+ apical to basolateral flux (J+ apical to basolateral flux (J+) of 3.3±0.2 pmol/cm²/h) significantly smaller than the basolateral to apical flux (J− apical to basolateral flux (J− apical to basolateral flux (J− apical to basolateral flux (J− apical to basolateral flux (J− apical to basolateral flux (J− apical to basolateral flux (J− apical to basolateral flux (J− apical to basolateral flux (J− apical to basolateral flux (J− apical to basolateral flux (J− apical to basolateral flux (J− apical to basolateral flux (J−) of 15.8±0.2 pmol/ cm²/h. Net secretion of digoxin was significantly inhibited by triiodothyronine (10μM) or estrogen-3-sulfate (10μM) at the basolateral membrane, or GF120918 at the apical membrane. Similar data was generated for canine PTC monolayers. These results were consistent with OATP4C1-mediated uptake of digoxin and apical efflux of digoxin mediated by MDR1. To test the utility of NHP monolayers as predictive models of nephrotoxicity, cells were challenged with a range of nephrotoxins using KIM-1 level, TEER, and cell viability as endpoints. In response to cisplatin challenge (10μM), KIM-1 release increased significantly alongside a significant decrease in cell viability to 34.4±4.4%, and TEER to 16.3±3.1 % of control values respectively. In addition to cisplatin-induced toxicity, a significant increase in KIM-1 release was found in response to challenge with 10μM cyclosporin, 10μM methotrexate, 200μg/ml polymyxin B or 200μg/ml gentamicin. Both cyclosporin and gentamicin had no effect upon either TEER or cell viability. Methotrexate and polymyxin B challenge resulted in a significant fall in both TEER and cell viability. These results report the successful production of primary NHP and canine PTC platforms which, similar to our human and rat aProximate™ PTC models, may prove useful as predictive in vitro PTC models of drug transport and drug toxicity.

2819 Preventive Effect of Sulforaphane on Type 2 Diabetes-Induced Diabetic Nephropathy via AMPK-Mediated Glucose/Lipid Metabolism Improvement

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Diabetic nephropathy (DN) is the leading cause of end-stage renal failure worldwide. Sulforaphane (SFN) was reported to prevent DN along with the activation of nuclear factor erythroid-2 related factor 2 (Nrf2) and AMP-activated protein kinase (AMPK). Dyslipidemia plays a crucial role in DN in type 2 diabetes (T2D)-induced DN. AMP-activated protein kinase (AMPK), particularly AMPK-α2 isoform, can attenuate the nephropathic alterations by improving lipid metabolism. To study if SFN can protect T2D-induced DN by activating AMPKα2 and Nrf2-mediated anti-oxidative pathways, male C57BL/6J and AMPKα2 knockout (AMPKα2-KO) mice were fed high-fat diet (HFD) for 3 months to induce T2D and treated with single or multiple short-term treatment with sulforaphane (STZ) to hyperglycemia as a T2D mouse model. Control mice were fed with normal diet without STZ. T2D and control mice were treated with or-with
suggesting that the MCLR-induced glomerular change was attenuated in HHFC-induced NASH. In contrast, 30 µg/kg MCLR increased casts and proteinuria in both control and HHFC, but to a greater magnitude in HHFC, suggesting that HHFC animals were more susceptible to these toxicities. The MCD group was refractory to MCLR-induced renal histopathological changes. Compared to the vehicle treatment within each diet, the 30 µg/kg MCLR dose decreased PP2A protein levels in both NASH groups, whereas the 10 µg/kg MCLR dose decreased PP2A protein levels only in the MCD group. MCLR bound to protein was detected only in the 30 µg/kg dose and is mostly observed at ~25-kDa in the control and HHFC groups, whereas in the MCD group most is observed at ~35-kDa. Control animals adapted to repeated MCLR exposure by increasing the urinary excretion of MCLR and MCLR-cysteine at the end of the study, whereas this adaptation was attenuated in HHFC animals. These data indicate that the mechanisms of altered MCLR-induced proteinuria and casts in HHFC-induced NASH may be associated with differential MCLR urinary elimination and modulation of PP2A and apoptosis. Collectively, these data suggest that NASH patients may be more susceptible to certain aspects of MCLR-induced renal toxicity and may contribute to the burden of chronic kidney disease in NASH patients. Funding: 4RO00E520455.

2820 Cisplatin Renal Cytotoxicity in Human Proximal Tubular Epithelial Cells Is Modified by Resveratrol Protection of Mitochondrial Function

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Cisplatin is a cancer chemotherapeutic agent used in treating testicular, ovarian, and cervical cancer. Cisplatin usage is associated with adverse effects that include neurotoxicity and nephrotoxicity. The frequency of these serious adverse effects increases with dose and cycles of treatment. Resveratrol (RES) is a polyphenolic compound that promotes apoptosis in some cancer cell lines and reduces tumor size in clinical studies. Part of the mechanism for reducing cisplatin toxicity by RES may be mediated by preserving mitochondrial function and diminishing cisplatin induced oxidative stress. Cisplatin targets renal proximal and distal tubules. HK-2 cells were selected for this study as they are a human renal proximal tubular epithelial cell line (HK-2). Our hypothesis is that RES will diminish mitochondrial impairment by cisplatin in HK-2 cells. HK-2 cells were plated for 48 h followed by a 1 h pretreatment with RES or 1% DMSO (vehicle). Renal cells were subsequently exposed to cisplatin at a final concentration of 0.50 µM for 24 h. Viability was assessed using MTT and trypan blue exclusion cell counts. All studies were conducted a minimum of 3 independent experiments. Cells pretreated with 10 µM RES were protected from cisplatin cytotoxicity at 24 h. Cisplatin caused a concentration dependent decline in MTT viability after 24 h exposure to 0.75 and 1.5 µM cisplatin. RES pretreatment for 1 h with 10 or 15 µM RES increased HK-2 viability relative to cells exposed only to RES Vehicle (DMSO). Additional studies examined whether RES stimulated cell growth as part of the mechanism for cellular protection. RES did not increase cell number as part of its protective mechanism. Mitochondrial function was evaluated at 24 h using a Seahorse XFp analyzer. Basal and maximal mitochondrial respiration were monitored in RES and cisplatin treated cells. Western analysis detected an increase in protein carbonylation with 24 h cisplatin exposure which was reversed by RES. Mitochondrial function was diminished by cisplatin and partially reversed by RES. RES protects human epithelial cells from cisplatin cytotoxicity, preserves mitochondrial integrity and mitochondrial respiration. Supported by NIH Grant INBRE R20GM103434 and a WV NASA Undergraduate Research Fellowship to MD and RM.

2821 Microcystin-LR Renal Toxicity in Nonalcoholic Fatty Liver Disease

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Non-alcoholic fatty liver disease (NAFLD), an advanced stage of NASH, nonalcoholic steatohepatitis (NASH), increases systemic exposure to microcystin-LR (MCLR), potentially increasing kidney exposure and toxicity. We hypothesize that MCLR will exacerbate renal toxicity in NASH. Sprague Dawley rats were fed either a control diet, a methionine or choline deficient (MCD) diet, or a high fat/high cholesterol diet (HHFC) (NASH) for 10 weeks. After 6 weeks of diet, animals were administered either vehicle, 10 µg/kg, or 30 µg/kg MCLR via i.p. injection every other day for 4 weeks. MCLR-induced NASH increased the glomerular change histopathology score (control=0.0±0.0, HHFC=1.0±0.0). After 30 µg/kg MCLR exposure, this score increased in both control and HHFC groups to ~1.5, sug-

2822 3D NephroScreen: High-Throughput Drug-Induced Nephrotoxicity Screening on a Proximal Tubule-on-a-Chip Model


Renal toxicity remains a major issue in clinical trials, and stresses the need for more predictive models fit for implementation in early drug development. Here, we describe a perfused, leak-tight renal proximal tubule cell (RPTec) model cultured within a high throughput microfluidic platform (Mimetas' OrganoPlate®), along with recent results from a 12-compound nephrotoxicity screen performed within the "NephroTube" CRACK IT consortium in collaboration with sponsors. Human monolayer, RPTec cells (Sigma) were grown against a collagen I ECM in a 3-channel OrganoPlate®, yielding access to both the apical and basal side. Drug-induced toxicity was assessed by exposing kidney tubules to 4 benchmark and 8 blinded compounds with known clinical effects supplied by the sponsors for 24 and 48h. The tightness of the barrier was evaluated by diffusion of a dextran dye from apical to basal compartment. Parallel to this, cell viability with a WST-8 assay and the presence of LDH in the supernatant were assessed. Finally, kidney tubules were lysed, and RNA was extracted for gene expression analysis of acute kidney injury markers. Upon perfusion flow, RPTec form leak-tight confluent tubular structures against the collagen I ECM in the OrganoPlate®. The NephroScreen revealed significant decreases in barrier tightness and cell viability of 7 out of 12 compounds. Furthermore, the release of LDH was significantly increased in 9 out of 12 compounds. An increased expression in HMOX1, TNFα and NGAL compounds. Furthermore, the NephroScreen revealed significant decreases in barrier tightness and cell viability of 7 out of 12 compounds. Furthermore, the release of LDH was significantly increased in 9 out of 12 compounds. An increased expression in HMOX1, TNFα and NGAL. The NephroScreen revealed significant decreases in barrier tightness and cell viability of 7 out of 12 compounds. Furthermore, the release of LDH was significantly increased in 9 out of 12 compounds. An increased expression in HMOX1, TNFα and NGAL. The NephroScreen revealed significant decreases in barrier tightness and cell viability of 7 out of 12 compounds. Furthermore, the release of LDH was significantly increased in 9 out of 12 compounds. An increased expression in HMOX1, TNFα and NGAL. The NephroScreen revealed significant decreases in barrier tightness and cell viability of 7 out of 12 compounds. Furthermore, the release of LDH was significantly increased in 9 out of 12 compounds. An increased expression in HMOX1, TNFα and NGAL. The NephroScreen revealed significant decreases in barrier tightness and cell viability of 7 out of 12 compounds. Furthermore, the release of LDH was significantly increased in 9 out of 12 compounds. An increased expression in HMOX1, TNFα and NGAL. The NephroScreen revealed significant decreases in barrier tightness and cell viability of 7 out of 12 compounds. Furthermore, the release of LDH was significantly increased in 9 out of 12 compounds. An increased expression in HMOX1, TNFα and NGAL. The NephroScreen revealed significant decreases in barrier tightness and cell viability of 7 out of 12 compounds. Furthermore, the release of LDH was significantly increased in 9 out of 12 compounds. An increased expression in HMOX1, TNFα and NGAL. The NephroScreen revealed significant decreases in barrier tightness and cell viability of 7 out of 12 compounds. Furthermore, the release of LDH was significantly increased in 9 out of 12 compounds. An increased expression in HMOX1, TNFα and NGAL. The NephroScreen revealed significant decreases in barrier tightness and cell viability of 7 out of 12 compounds. Furthermore, the release of LDH was significantly increased in 9 out of 12 compounds. An increased expression in HMOX1, TNFα and NGAL. The NephroScreen revealed significant decreases in barrier tightness and cell viability of 7 out of 12 compounds. Furthermore, the release of LDH was significantly increased in 9 out of 12 compounds. An increased expression in HMOX1, TNFα and NGAL. The NephroScreen revealed significant decreases in barrier tightness and cell viability of 7 out of 12 compounds. Additionally, the NephroScreen has revealed the ability to detect drug-induced toxicity in the context of proteinuria, which is a biomarker for chronic kidney disease progression. The NephroScreen has also shown the ability to detect drug-induced toxicity in the context of proteinuria, which is a biomarker for chronic kidney disease progression. The NephroScreen has also shown the ability to detect drug-induced toxicity in the context of proteinuria, which is a biomarker for chronic kidney disease progression. The NephroScreen has also shown the ability to detect drug-induced toxicity in the context of proteinuria, which is a biomarker for chronic kidney disease progression. The NephroScreen has also shown the ability to detect drug-induced toxicity in the context of proteinuria, which is a biomarker for chronic kidney disease progression.
2824 Circulating microRNA Biomarkers for Drug-Induced Tubular and Glomerular Injury in Rats
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Acute kidney injury is an important clinical concern. For the early phase detection, the present study focused on circulating microRNA (miRNA) as a novel biomarker for kidney injury. Through next-generation sequencing analysis, we aimed to identify specific plasma miRNAs that can enable prediction and discrimination of tubular and glomerular injuries. Six-week-old male Sprague-Dawley rats were intravenously administered cisplatin (CSP) and gentamicin (GEN) to induce tubular injury. To make glomerular injury models, uranyl oxycromic (PUR) and doxorubicin (DOX) were intravenously administered. Small RNA-sequencing was performed to analyze time-dependent changes in the plasma miRNA profiles. The sequence data was firstly mapped to the mature miRNA sequences of rats. The sample normalization was performed with DESeq package of R software. Considering that the PUR- and DOX-induced glomerular injury models were accompanied by tubular injury, the specifically changed miRNAs among PUR and DOX models were defined as glomerular injury specific miRNAs. In the differential analysis, we focused on miRNAs with following criteria: (1) |log2(ratio)| > 2 and FDR < 0.05; (2) the earlier responses to the kidney damage, and (3) the level of changes in the normalized read counts. Through the analysis, several miRNAs such as miR-148a-3p and miR-143-3p were commonly down-regulated in all of the models, and miR-122-3p and miR-192-5p were specifically down-regulated in the glomerular injury models. Our data indicated that the combination of several specific miRNAs in the plasma may enable the early and sensitive detection of tubular and glomerular injuries. The present study suggests the potential utility of plasma miRNAs in the early and type-specific detection of kidney injury.

2825 Modeling the Effects of Proteinuria on a Human Renal Proximal Tubule Using a 3D Microphysiological System
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Proteinuria is a pathological cause of progressive renal impairment in chronic kidney disease. Changes in ultrafiltrate composition during glomerular dysfunction may promote or exacerbate tubulointestinal lesions by activating pro-inflammatory, pro-fibrotic, and vaso-modulatory signaling in proximal tubule epithelial cells (PTECs). To better understand how serum-filterate components drive PTECs toward an activated phenotype, we treated a 3-dimensional microphysiological model of the human renal proximal tubule with human serum. Exposure of PTECs to 2% human serum for 48 hours increased secretion of kidney injury molecule 1 (KIM-1) and expression of proliferation marker protein Ki-67 (K167), both of which are events that occur during tubular wound healing. In vivo. Transcriptomic profiling by RNA-seq revealed the expression of 820 genes were significantly affected. Ingenuity Pathway Analysis software identified transforming growth factor beta (TGF-β) and tumor necrosis factor alpha (TNFα) as the primary upstream regulators mediating the observed transcriptional changes. Many of the affected genes have roles in matrix remodeling, immune cell modulation, and endothelial activation. These data suggest that our microphysiological system reproduces the pathophysiological response of the proximal tubule to serum and has utility in exploring the mechanisms of PTEC activation as well as potential therapeutic interventions. Future efforts include evaluating the impact of inhibiting TNFα or SMAD signaling on PTEC response to human serum.

2826 Time-Dependent Regulation of Calbindin Expression in Cisplatin Kidney Injury

Calbindin is a cytosolic calcium-binding protein expressed in distal tubules and collecting ducts of the nephron. Since the discovery that calbindin is released into urine after kidney injury, there has been growing interest in using calbindin as an early and sensitive biomarker of nephrotoxicity. However, very little is known about the intrarenal regulation of calbindin turnover. We therefore sought to characterize the time-dependent regulation of renal calbindin release and expression in the kidneys during cisplatin nephrotoxicity, a well-established model of acute kidney injury. Male C57BL/6J mice were treated with vehicle or cisplatin (20 mg/kg, i.p.), and urine, plasma, and kidneys collected at 24-hour time points. Urinary calbindin and kidney injury molecule-1 (Kim-1) concentrations were elevated by 11.6-fold and 2.5-fold, respectively by day 2. Serum creatinine and blood urea nitrogen, traditional markers of renal injury, increased in cisplatin-treated mice by day 3, confirming the presence of acute kidney injury. Concurrently, time-dependent decreases in intrarenal calbindin protein were observed on days 3 and 4. Additionally, a 200-fold up-regulation of both calbindin (Calb1b) and Kim-1 miRNA was seen on day 3. These data suggest that early release of calbindin from the kidneys into the urine initiates the compensatory induction of miRNA expression at later time points (days 3 and 4). Interestingly, Calb1b up-regulation paralleled an increase in the mRNA expression of the apical TRPV5 calcium channel and a decrease in the basolateral sodium-calcium exchanger NCX1. These data suggest a coordinated response to modulate ion and calcium transport, which may be required to maintain renal integrity. In summary, an improved understanding of the regulation of renal calbindin during cisplatin nephrotoxicity enhances its utility as a potential biomarker of kidney damage. Supported by P30ES050522 and T32ES007148.

2827 Effects of the SGLT-2 Inhibitor Canagliflozin on Adenine-Induced Chronic Kidney Disease in Rats
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SGLT-2 inhibitors have been shown to be nephroprotective in diabetes. Here, we examine if one of these drugs could also ameliorate non-diabetic chronic kidney disease (CKD). Six groups were used: control, adenine-fed, and canagliflozin (10 or 25 mg/kg, by gavage), each with or without adenine. Rats fed adenine showed the typical features of CKD that included elevation of blood pressure, decreased food intake and growth, increased water intake and urine output, decrease in creatinine clearance, and increase in urinary albumin/creatinine ratio, liver-type fatty acid binding protein, N-acetyl-beta-D-glucosaminidase, and plasma urea, creatinine, uric acid, calcium, indoxyl sulfate and phosphorus concentrations. Adenine also increased concentrations of several biomarkers of inflammation such as neutrophil gelatinase-associated lipocalin, interleukin-6, tumor necrosis factor alpha, clusterin, cystatin C and interleukin-1β, and decreased some oxidative biomarkers in kidney homogenate, such as superoxide dismutase, catalase, glutathione reductase, total antioxidant activity, and urinary 8-isoprostane and urinary 8-hydroxy-2-deoxyguanosine. Adenine significantly increased the renal protein content of Nrf2, caused renal tubular necrosis and fibrosis. Given alone, canagliflozin at the two doses used did not significantly alter any of the parameters mentioned above. When canagliflozin was given concomitantly with adenine, it significantly and dose-dependently ameliorated all the measured adenine-induced actions. Canagliflozin ameliorated adenine-induced CKD in rats, through reduction of several inflammatory and oxidative stress parameters, and other indices of acute toxicity. Moreover, the increase in the renal content of the transcription factor Nrf2. The drug caused no overt or significant untoward effects, and its trial in patients with CKD may be warranted.

2828 The Regulation of Mitochondrial Fusion in Cold Storage Kidney Preservation

A major hurdle in the field of renal transplantation is the shortage of suitable donor kidneys. A large gap remains between graft survival of renal allografts from deceased donors versus living donors. Kidneys from deceased donors...
must undergo cold preservation before transplantation to allow time to find a suitable recipient. Even though cold storage greatly extends the window of opportunity for transplanting kidneys, it also leads to mitochondrial injury impairing overall graft outcome. The goal of this study is to investigate the role of key proteins that regulate the mitochondrial fusion machinery during cold storage injury using an in vitro kidney transplant model. In our studies we show that key mitochondrial proteins (OMA1, YME1L, and OPA1) involved in mitochondrial fusion machinery are impaired after cold storage. Furthermore, we show that OMA1 knockdown combined with cold storage restores OPA1 processing suggesting that OMA1 loss may have a protective effect against cold storage induced mitochondrial injury by restoring normal fusion. We also show that OMA1 sRNA increases in ATP-dependent cell viability during cold storage. Additionally, our lab has developed a novel assay for measuring OMA1 enzymatic activity using fluorescence resonance emission technology. Taken together, this data suggest that OMA1 may be a promising therapeutic target for improving the function of kidneys transplanted after cold storage exposure.

2829 Cytotoxicity, Mitochondrial Function, and Endoplasmic Reticulum (ER) Stress Associated with the Radiocontrast Agent Diatrizoate (DA) in a Human Proximal Tubular Cell Line

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Contrast-Induced Acute Kidney Injury (CI-AKI) is the third most common cause of hospital associated kidney damage. Contrast agents are necessary for many diagnostic procedures such as arteriography, venography, and whole body CAT scans. The mechanisms of contrast-induced renal impairment are not entirely known but oxidative stress, diminished renal hemodynamics, and direct cytotoxicity have all been hypothesized. The hypothesis for this study is that diatrizoate (DA) induces direct cytotoxicity to human proximal tubule epithelial (HK-2) cells via oxidative stress, diminished mitochondrial function, and induced endoplasmic reticulum (ER) stress. HK-2 cells were plated for 48 h to equilibrate followed by a 24 h exposure to DA (0-30 mg/mL). All studies were run with a minimum of 3 independent experiments. Viability was measured at the end of the 24 h period using Cell Countess Trypan Blue exclusion and MTT conversion to formazan. Oxidative stress and ER stress were monitored using Western blot analysis for protein expression. Mitochondrial function was monitored using an Agilent Seahorse analyzer. Agilent Seahorse Cell Mito Stress Tests and Cell Glycolysis Stress Tests were performed on HK-2 cells exposed for 24 h to DA. Diminished cell function was evident within 24 h beginning with 2 mg I/mL DA as measured by the MTT assay. Western blot analysis indicated a rise in protein carbonylation as a biomarker of oxidative stress following DA exposure relative to vehicle (p<0.05). Biomarkers for ER stress included a rise in caspase 12 in the DA group relative to vehicle. Basal Oxygen Consumption (OCR) and maximal OCR were diminished by 15 mg I/mL DA relative to control (p<0.05). DA impaired HK-2 cells by inducing a rise in oxidative stress and diminishing mitochondrial function. Supported by NIH INBRE BP20GM10343 and a WV NASA Graduate Fellowship to DW.

2830 Confirmed Biphenyl-Dependent Formation of Calculi Observed in SD Rats Have Been Shown to Initiate Bladder Tumors in Rodents Only

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Biphenyl (BP) is an aromatic chemical used as a synthesis intermediate and in heat transfer fluids. The rich toxicological dataset, with a number of rodent companies, suggests that diatrizoate (DA) induces direct cytotoxicity to human proximal tubule epithelial (HK-2) cells via oxidative stress, diminished mitochondrial function, and induced endoplasmic reticulum (ER) stress. HK-2 cells were plated for 48 h to equilibrate followed by a 24 h exposure to DA (0-30 mg/mL). All studies were run with a minimum of 3 independent experiments. Viability was measured at the end of the 24 h period using Cell Countess Trypan Blue exclusion and MTT conversion to formazan. Oxidative stress and ER stress were monitored using Western blot analysis for protein expression. Mitochondrial function was monitored using an Agilent Seahorse analyzer. Agilent Seahorse Cell Mito Stress Tests and Cell Glycolysis Stress Tests were performed on HK-2 cells exposed for 24 h to DA. Diminished cell function was evident within 24 h beginning with 2 mg I/mL DA as measured by the MTT assay. Western blot analysis indicated a rise in protein carbonylation as a biomarker of oxidative stress following DA exposure relative to vehicle (p<0.05). Biomarkers for ER stress included a rise in caspase 12 in the DA group relative to vehicle. Basal Oxygen Consumption (OCR) and maximal OCR were diminished by 15 mg I/mL DA relative to control (p<0.05). DA impaired HK-2 cells by inducing a rise in oxidative stress and diminishing mitochondrial function. Supported by NIH INBRE BP20GM10343 and a WV NASA Graduate Fellowship to DW.

2831 Effect of 4-Methylpyrazole on Acetaminophen-Induced Hepatotoxicity

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Acetaminophen (APAP) hepatotoxicity is the leading cause of acute liver failure in the United States, and a significant proportion of APAP overdose patients also develop hepatotoxicity. While mechanisms of APAP-induced liver injury have been well studied, molecular mechanisms involved in APAP-induced kidney injury are not well characterized. We have previously demonstrated that 4-methylpyrazole (4MP), an US FDA approved antidote against methanol and ethylene glycol poisoning, can provide significant protection against APAP-induced liver injury by inhibition of cytochrome P450-mediated generation of the reactive APAP metabolite, N-acetyl-p-benzoquinone imine (NAPQI). To determine the kidney protective effect of 4MP, we used a chemical model of acute kidney injury induced by cytochrome P450, this study explored whether 4MP could also prevent APAP-induced kidney damage and thus have a dual benefit in APAP overdose. Male C57BL/6J mice were treated with 300mg/kg APAP with or without 4MP for 2, 6 or 24h, followed by analysis of APAP metabolites (2h) and cell injury (6 and 24h) within the liver as well as kidney. Western blots for cytochrome P450 2E1, demonstrated comparable enzyme expression in both livers and kidneys, whereas APAP-protein adduct measurement indicated significantly elevated levels in the liver when compared to the kidney. Treatment with 4MP essentially eliminated adduct levels in both organs. Analysis of APAP metabolites at the 2h time point indicated that 4MP inhibited formation of APAP-GSH, APAP-NAC and APAP-Cys in the liver, while APAP-Cys was significantly decreased in the kidney. However, APAP-sulfate levels were increased by 4MP treatment in the kidney. Interestingly, estimation of JNK activation, which is a hallmark of APAP-induced liver injury, indicated no activation in kidney, while the liver showed robust activation, which was inhibited by 4MP as expected. In conclusion, the data suggests that there is appreciable expression of cytochrome P450 in the kidney and some APAP metabolism does occur in the organ. In addition to its effect in the liver, 4MP treatment can also inhibit the oxidative metabolism of APAP in the kidney. However, in contrast to the liver, the signaling mechanism of cell injury in the kidney may be independent of JNK.

2832 Effect of Levosimendan, an Inodilator, on Cisplatin-Induced Nephrotoxicity in Rats


Levosimendan is a positive inotropic agent with vasodilating properties. It increases the sensitivity of troponin C to calcium in cardiac cells, causes vaso-dilation by opening the (ATP)-sensitive potassium channels in smooth muscle cells and possesses anti-inflammatory and antiapoptotic effects. We investigated the effect of levosimendan on cisplatin (CP)-induced nephrotoxicity in rats. Rats were randomly divided into four groups (n = 6). The first and second groups received normal saline (control) and intraperitoneal (i.p.) cisplatin (6 mg/kg) on day 7, respectively. The third and fourth groups were given a single intraperitoneal (i.p.) injection of CP on day 7 and levosimendan (1 mg/kg/day) or vehicle for 10 days, respectively. At day 11, animals were anaesthetized and blood collected and kidneys removed. Another four groups were treated the same as the previous four groups to measure renal blood flow. CP significantly increased plasma urea, creatinine and neutrophil gelatinase-associated lipocalin (NGAL) levels. In addition, CP increased urinary albumin/creatinine ratio, N-Acetyl-β-D-Glucosaminidase (NAG) activity and reduced creatinine clearance. CP also significantly increased the plasma concentration of plasma tumor necrosis factor-α (TNF-α) and significant changes in reduced antioxidant indices (catalase and superoxide dismutase (SOD)) and increased malondialdehyde (MDA) levels. Histopathologically, CP caused remarkable renal damage compared with control. Moreover, CP reduced renal blood flow. Levosimendan significantly ameliorated CP-induced biochemical, histopathological and hemody-
**Analytical Methods Impact Estimates of TCE’s GSH Conjugation and Risk Assessment**


Trichloroethylene (TCE) is enzymatically conjugated with glutathione (GSH) to form S-(1,2-dichlorovinyl)-glutathione (DCVG), which is further metabolized to 5,6-dichloro-1,2-dihydroxyacetylenic (DHA) and dichloroacetylglutathione (DCVC). These GSH conjugates have been implicated in kidney toxicity and kidney cancer in rats from TCE exposure. Studies have reported very different levels of these metabolites in subcellular fractions and tissues, depending on the method used: HPLC-UV (high performance liquid chromatography-ultraviolet) or HPLC-MS (HPLC-mass spectrometry), with values being much higher for the HPLC-UV method. To examine these differences, spiked concentrations of DCVG and DCVC were measured side-by-side in rat and human tissues using HPLC-UV or HPLC-MS/MS (HPLC-Tandem MS). Following derivatization of DCVG and DCVC with dimethyl sulfoxide (DNS), DCVC eluted at the solvent front with the HPLC-UV method and could not be quantified. DNS-DCVG, however, was quantified in rat kidney, heart, and liver, but not in human kidney, heart, or liver. Collagen IV, fibronectin, and PCNA increased in kidneys of 4-week-old and 36-week-old female OVE26 mice, while the protein levels of PCNA increased in kidneys of both male and female OVE26 mice, but the protein levels of p53 were approximately two-fold higher in male OVE26 mice compared to female OVE26 mice. In addition, the mRNA and protein expression levels of apoptosis-related markers were significantly increased from those of individual treatment. To further elucidate the mechanism of combined toxicity in kidney and liver, both quantitative real-time PCR and Western blot were applied to confirm mRNA and protein expression levels of DCVG and DCVC in both kidney and liver. In this study, we investigated the combined toxicity of OTA and AA in the kidney and liver cells. The cell viability with the treatment of OTA and AA was checked to determine the concentration at which the synergistic effect occurs between OTA and AA. The toxicity of the combination of both substances showed synergistic effects regarding cell viability and ROS production. In addition, mRNA and protein expression levels of apoptosis-related markers were significantly increased from those of individual treatment. To further elucidate the mechanism of combined toxicity in kidney and liver, both quantitative real-time PCR and Western blot were applied to confirm mRNA and protein expression levels of DCVG and DCVC in both kidney and liver. The mRNA and protein expression levels of collagen IV, fibronectin, and PCNA were significantly increased from those of individual treatment. 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which are either publicly available (e.g., the NIST/EPA/NIH Mass Spectral Library) or private/custom-built. Further, the action of making a “positive” match of an identity to a detected substance relies on expert judgement, which often incorporates database search software and scoring algorithms. While definitions may be found in USP 1663 for the various levels of confidence in identification (e.g., tentative, confident, and confirmed), such terminology is not uniformly applied and, therefore, open to interpretation. The goal of the current research is to assess the results of identification of untargeted analysis in one testing scenario. Samples of 0.25% ZDBC in PVC and HDPE were extracted at 6 and 12 cm²/mL for 24 h at 37 °C and 50 °C in isopropanol. An Agilent Technologies 7890B GC/MS was used to analyze 1 µL injection volumes of samples, utilizing a DB-5MSUI column, and heating profile of 50 °C (3 mins), then 12 °C/min until 315 °C, followed by a 15 min hold. Analysis was conducted using Agilent MassHunter Unknows Analysis software version B.08.00. Peaks were detected using deconvolution mode and searched in the NIST 1A v17 library with scores adjusted to match the NIST dot product match factor algorithm. Samples were blank subtracted, and then manually curated to remove hits not considered true peaks or that were below the reporting threshold (500 ppb). A 500 ppb mix of seven reference standards was utilized, and the lowest response factor (RF) of the seven was used as the manual RF. Reference standard RFs ranged by 28-fold, indicating high response factor variability. The average number of identification matches within 10 points of the true identity of the reference standards ranged from 1 to 7 and averaged 3.3. Of 36 samples analyzed, 15 contained extractable substances above the reporting threshold, containing an average of 1.6 +/- 0.5 substances at levels of 0.79 +/- 0.13 µg/mL. The average match score for the most abundant substance in a sample was 83 +/- 3 with generally 7 or more alternative identifications within 10 match factor points of the top candidate. These results illustrate a potential for variability in substance identification and will be addressed by additional studies.

2839 Derivation of Short-Term Threshold of Toxicological Concern Values for Extractable Chemicals from Medical Devices

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The increased reliance on chemical characterization and risk assessment for assessing the potential toxicological risks from medical devices and materials necessitates the use of Threshold of Toxicological Concern (TTC) values to prioritize the evaluation of extractable chemicals. The TTC approach is based on the concept that chemical structures can define the potential for toxicity, and that common structural features can be used to group substances into various categories of toxicological concern. Current TTC values derived for lifetime exposure are overly conservative when used to evaluate the potential for systemic health hazards associated with limited (<24 hours) and prolonged (>24 hours and <30 days) exposure durations, leading to an overestimation of the relevant risks. To investigate the methodology for short-term TTC derivation, a database for short-term toxicity was produced from extractable chemicals commonly identified in medical devices. Organic chemicals with structural alerts or data suggestive of carcinogenicity were excluded, and systemic effect levels were identified from multiple endpoint subacute, subchronic, and reproductive toxicity studies. Developmental effects were investigated as a separate descriptor. Tolerable intakes (TI) were derived in accordance with ISO 10993-17 and then fit to a distribution curve, from which 5th percentile values were calculated for limited and prolonged exposures. The derived TI values were discordant with the anticipated Cramer classification (ToxTree, Version 3.1.0), and similar thresholds of toxicity were observed for both class I and III chemicals. Variation among the derived TI values for Cramer I chemicals suggests the need for additional refinement of the predicted toxicity for this class. The results overall suggest the ability to integrate structural knowledge of extractable chemicals from medical devices into a threshold evaluation approach to prioritize the evaluation of potential systemic health risks for short-term exposure to these chemicals.

A Case Study: Solvent Extract Analysis as a Way to Bridge Biocompatibility Data for a Material Change

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ISO 10993 requires test article extracts to be observed pre- and post-extraction for color changes, presence of particulates and test article degradation. It is not uncommon to observe particulates in the extract sample. Current standards (USP 788 and AAMI TIR42) address particulates evaluation associated with intravenous medical device only. US FDA Color Additive (CA) Policy requests supplemental CA information if the extract has particulates. This case study gives an example of a new device biological evaluation when particulates found in extracts. A given device is used for saline (NS) irrigation, and classified as an externally communicating device in contact with tissue, bone and dentin for < 24 hrs. The test article polar (NS) and non-polar (sesame oil) extracts (50°C for 72 hrs) were tested for cytotoxicity (37°C for 24 hrs MEM extract only), sensitization, irritation and acute systemic toxicity per ISO 10993 with acceptable results. However, orange particulates were in all NS extracts (the origin was identified to be from a 303 stainless steel (SS) component), and white particulates were in some sesame oil extracts. Orange particulates were identified by scanning electron microscope to be iron/chromium/oxygen, and were caused by oxidation. White particulates were identified by infrared spectroscopy to be polycarbonate, which was attributed to the sample cutting process. Oxidation was also observed in clinically relevant condition (NS, 37 °C for 12 hrs), which was attributed to the machining process. Agitation options on 303 SS were investigated to improve its corrosion resistance, but was not an effective alternative. A material change from 303 SS to 304 SS was then proposed and evaluated. No oxidation on 304 SS components was observed for the NS extraction up to 72 hrs at 37 °C. No further biocompatibility testing was performed on the finished product following the material change. The US FDA 510(K) was cleared based on the initial biocompatibility testing (303 SS), particulates identification, and corrosion testing of 304 SS in a simulated-use extraction. No CA information was required when the identified particulates were not CA related. This case study indicates using chemical analysis to bridge biocompatibility data for a material change is well accepted by US FDA. It saves time, cost, and animals for device evaluation.

Establishing a Tolerable Intake Level for a Medical Device Extractable Using Methyl Isobutyl Ketone as a Surrogate

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As specified in ISO 10993-17:2002, “The determination of the suitability of a medical device for a particular use involves balancing any identified risks with the clinical benefit to the patient associated with its use. Among the risks to be considered are those arising from exposure to leachable substances arising from medical devices.” After tert-butyl isopropyl ketone (TIBK) was detected during extraction testing of a permanent implant, a risk assessment was performed to calculate a tolerable intake (TI). A TI is defined as the estimate of the average daily intake of a substance over a specified time period that is considered to be without appreciable harm to health. As no chemical-specific toxicity data were identified for TIBK, the structurally similar methyl isobutyl ketone (MIKB) was identified as a potential surrogate. ToxMatch calculated a Tanimoto coefficient of 0.9 between the target compound and MIKB, which above the cutoff of 0.6 and indicates that TIBK is structurally similar to MIKB. Therefore, chronic toxicity data on MIKB were used to derive a TI for TIBK. A number of tumors were reported in rats and mice following long-term inhalation exposure to MIKB. The kidney tumors in male rats are likely associated with α2μ-globulin toxicity, and liver neoplasms in male and female mice are likely the result of constitutive androstane receptor (CAR) activation, discounting their use in derivation of a TI for medical device applications. Although a positive trend in the increased incidence of mononuclear cell leukemia in rats was reported following chronic exposure to MIKB, statistical significance was only reached at the highest dose group; this neoplasm is common in mouse control groups, and the strength of response was too weak for NTP to conclude that it was associated with MIKB exposure. Therefore, TIBK was evaluated as a non-carcinogen using a NOAEL of 1,026 mg/m³ that was used to derive the US EPA’s MIKB reference concentration. Using default parameters in ISO 10993-17:2002 for an inhalation rate of 20 m³/day and default body weight of 70 kg, MIKB’s NOAEC, was converted to a solid dose of 293 mg/kg/day. Applying uncertainty factors to account for inter- and intraspecies variability, inhalation to solid dose, and use of a surrogate yields a TI of 2,442 mg/kg/day for TIBK.
2941 Risk Assessment of Leachable Substances from a Drug Coated Balloon Catheter Classified as a Combination Product


A case study is presented for a toxicological risk assessment for a drug coated balloon catheter. The device is classified as a combination product under the US FDA Office of Combination Products as subject to the safety requirements for medical devices (under CDRH) and drug products (under CDER). Due to the complexity of issues that can arise, the overall study design required early coordination among toxicologists, chemists, and US FDA. The device is used in angioplasty, to provide mechanical dilation of arteries, while the drug coating provides an ancillary effect of reducing restenosis. Therefore, the risk assessment evaluated potential exposure to leachables arising from the device as well as impurities/degradation related to the drug component (which is released and absorbed onto the artery walls). An extractables analysis was conducted separately for the drug-coated balloon and the uncoated balloon. Ex Extractions were conducted with purified water, 50% isopropyl alcohol, and hexane at 50K Number 8728C for an exhaustive duration. The total extractable amount of each substance was viewed as an estimate of a one-time exposure. Exposure estimates were compared to a Threshold of Toxicological Concern (TTC), which eliminates substances present below levels of any toxicological concern. Substances above the TTC were compared to a Tolerable Exposure (TE) level derived from repeated dose animal studies. To avoid overly conservative risk estimates, a Proportional Exposure Factor, defined as in ISO 10993-17, was used to modify the TE, considering the differences between chronic and acute exposure. If a TE could not be derived for substances lacking published toxicological studies, exposures were compared to a modified TTC or a Qualification Threshold (QT) for impurities or degradants in drug products. Chemical structure was used to identify potential degradants. In summary, three toxicity thresholds (TTC, TE, and QT) were used to establish safety thresholds for leachable substances, and the benefits derived from therapeutic efficacy were considered when assessing drug product degradants.

2942 Role of Device Extraction Methods in Biocompatibility Assessment. Differential Effects of Latex in the Bacterial and Mammalian Test Systems


Safety assessment of medical devices usually involves collecting the extract per ISO 10993-12 and applying it to in vitro/in vivo test systems. Sometimes, the results are dependent on how the test samples are prepared during extraction as described in the following case studies. A vascular introducer Sheath, when tested for biocompatibility, showed particulates in the extract. So, the extraction procedure was repeated using intact (uncut) devices with no evidence of visible particulates. This shows that during normal clinical use the device is safe in patients and formation of particulates is an experimental artifact. In the second case, a cardiovascular catheter with stainless steel blade was evaluated for PITT. Two samples showed the time to form blood clot was 24% of negative control instead of the US FDA limit of > 80%. Upon further investigation, the failed test samples were found to contain tubing made with natural rubber latex. This latex component is in the proximal non-patient contacting portion of the device and was not supposed to be included in the biocompatibility testing. However, in the interest of patient safety, the catheter was further tested in an in vivo porcine model under simulated clinical use condition, thus confirming the lack of thrombogenic risk to patients. Finally, a nephrostomy catheter showed cytotoxicity test failure. But other in vivo assays such as irritation, sensitization, systemic toxicity and rabbit pyrogenicity have all showed passing data. During genotoxicity testing, both polar/non-polar extracts have passed the Ames bacterial mutagenicity assay. In contrast, there was a significant increase of mutation frequency in mouse lymphoma (L5178Y) cultures treated with RPMI extract in the absence of S9 but not in the presence of S9. A detailed examination of the study revealed that a latex cuff (located outside the patient’s body) was included in the extraction mixture which may have led to the positive mutagenic response. Repeating the mouse lymphoma assay without latex cuff confirmed the mutagenic response is indeed due to the presence of latex. Though the effects of natural rubber latex on cytotoxicity and coagulation are known, this is the first demonstration of the differential effects of latex on genotoxicity parameters. In summary, during biocompatibility assessment it is always better to ensure only patient contacting components are included and if necessary the extraction procedure is conducted under clinically relevant conditions.

2943 Proposal for the Application of Threshold Potency and Severity of Harm When Assessing Toxicological Risk of Non-Cancer Systemic Toxicity of Medical Device Constituents

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Toxicological threshold values are numbers derived by toxicological risk assessors to make informed judgments whether exposure to chemicals/compounds will present tolerable toxicological risk. Systemic toxicity threshold values are applied to chemicals/compounds with known or suspected systemic toxicity potential. We investigated whether potency and severity categories of non-cancer medical device chemicals/compounds might be useful when assessing the non-cancer systemic toxicological risk. Toxicological threshold values for medical device chemicals/compounds was obtained from the US EPA IRIS and ECHA Registered Substances databases. The toxicological thresholds were mined for non-cancer systemic oral subchronic/chronic toxicity thresholds. The potency of the selected toxicological thresholds were categorized as low (high potency) or high (low potency) based on the non-cancer threshold of toxicological concern (TTC) values of 1.5 (Cramer Class III) or 30 (Cramer Class I) micrograms per kilogram body weight per day, respectively. Severity of harm categories were defined as low (No observable functional change), intermediate (medical intervention not required), or high (medical intervention could be required). Results of our analysis are the following: (a) 86.6% - 93% of the chemicals have a toxicological threshold >0.03 mg/kg bw/day (Low Potency), (b) 2.1% - 5.4% of the chemicals have a toxicological threshold between 0.0015 and 0.03 mg/kg bw/day (Intermediate Potency), and (c) 9.9% - 8% of the chemicals have a toxicological threshold <0.0015 mg/kg bw/day (High Potency). All of the chemicals/compounds with a high potency toxicological threshold value were inorganics. Toxicological thresholds for organic chemicals/compounds were categorized as low (Cramer Class II) to intermediate (between Cramer Class I and III) potency. There was no difference in the number of Cramer Class I and II low potency organic chemicals/compounds categorized as low or intermediate severity. There were more low potency Cramer Class III chemicals/compounds categorized as high severity. Assigning non-cancer systemic toxicological thresholds into potency and severity categories may be useful for making expert judgments when conducting toxicological risk assessments of medical device chemicals/compounds.

2944 Predicting Exposure and Toxicity to Nickel Released from Cardiovascular Devices Using Multi-scale Modeling

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Many cardiovascular device alloys contain nickel, which if released in sufficient quantities, can lead to adverse health effects. However, in vivo nickel release from implanted devices and subsequent biodistribution of nickel ions to local tissues and systemic circulation are not well understood. To address this uncertainty, we developed a multi-scale (material, tissue, and system) biokinetic model. The model links nickel release from an implanted cardiovascular device to concentrations in serum and urine, which can be readily monitored, as well as in peri-implant tissue. The model was parameterized for a specific cardiovascular implant type, nitinol septal occluders, using in vitro nickel release test results, studies of ex vivo uptake into heart tissue, and in vivo and clinical measurements from the literature. Our results show that the model accurately predicts nickel concentrations in peri-implant tissue in an animal model and in serum and urine of septal occluder patients. The congruity of the model with these data suggests it may provide useful insight to establish nickel exposure limits and to interpret biomonitoring data. Additionally, we use the model to predict local and systemic nickel exposures during peri-implant device use. We find that nickel release per device surface area does not exceed 0.074 µg/cm² and is less than 32 µg/d in total. Finally, the local nickel exposure predictions can be utilized with in vitro cytotoxicity assessments to characterize cellular responses to expected in vivo concentration.
Biological evaluation of medical devices often includes chemical characterization (as described in ISO 10993-18). Device materials are subject to extraction where by materials, processing additives, residues, as well as material breakdown products may be released. These extractables have potential consequence on the biological response to the device during its use. Chemical characterization studies are followed up by toxicological risk assessments which evaluates potential harm from exposure to the extractables. In some cases, evaluation of material equivalence of similar device materials is required. Following extraction, chemical analysis is performed by various methods, such as GC/MS, FTIR, and LC/UV/MS. Current technology in high resolution mass spectrometry may improve these analyses. This presentation discusses development of improved data analysis methods to probe chemical analysis data from two materials’ equivalency. Two batches of two medical device materials, polyether block amide (commonly known as Pebax) and polypropylene, were analyzed. Multiple rounds of extractions (24 hour at 50°C 200 rpm) were used to obtain non-volatile residue. Test extracts were introduced to different instruments: a GC/MS (7890B/5977B Agilent) and an UHPLC-QTOF-MS system (6540 UHD- Agilent). Amounts of extractables were estimated semi quantitatively by comparing to a set of internal standards. Standards of various polymer additives were prepared and analyzed by LC-PDA-MS system where the UV absorbance and MS data were acquired together. Processed and raw Pebax materials were extracted by water and hexane, the total non-volatile residues on the order of 60 mg/g 5 g sample. GC/MS analysis revealed minor amounts of extractables (less than 20 μg/l 1 g sample). The GC/MS data were also processed for identification of analytes using the 2017 NIST Mass Spectral Library. Preliminary results showed more than 20 compounds tentatively identified. Ongoing work includes the use of Q-TOF MS with greater resolution (~40,000) for unknown identification using a commercial E&L Database. A scoring scheme for binary comparison of the data was utilized using Pearson correlation. The current results indicate Pearson correlation can be used to compare multi-dimensional data in binary steps. However more data mining is needed to ascertain material equivalency.

Materials to Probe Material Equivalency

L. Guy1, M. Assadi1, N. Jackson1, M. Chagnon1, F. Soza1, and G. Leclerc1,2
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Micro-computed tomography (Micro-CT) represents a non-destructive high-resolution imaging modality in which CT is performed on small samples such as explanted tissues. Typical image resolutions of 1-40μm/voxel can be generated and a three-dimensional (3D) model of the sample can be reconstructed. When applied to implanted medical devices, this technique can contribute to histology and histomorphometry of in vivo samples. It can fully evaluate the safety and performance of the medical device and to provide a full volume analysis rather than only limiting the analysis to the plane of the histology slide. The technology was applied to evaluate the resorption of magnesium implants. Yucatan miniswine coronary arteries implanted with magnesium scaffold were imaged by micro-CT and a thresholds band on density was applied to the images. Those thresholds were compared to specialized histology using Raman and infrared spectroscopy combined with X-ray diffraction analysis. Low-density material was then identified as magnesium hydroxide and high-density as intact magnesium. Volumetric evaluation of these two forms magnesium cage was performed using a commercial E&L Database. A scoring scheme for binary comparison of the data was utilized using Pearson correlation. The current results indicate Pearson correlation can be used to compare multi-dimensional data in binary steps. However more data mining is needed to ascertain material equivalency.

Correlation of Micro-CT Imaging with Histology Sections, an Important Tool for Understanding the Structural Function and Biological Response to Implantable Medical Devices

L. Guy1, M. Assadi1, N. Jackson1, M. Chagnon1, F. Soza1, and G. Leclerc1,2
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In Vitro Sensitization Assays for Medical Device Industry per ISO-10993-10: Challenges and Opportunities

B. R. Alzuia, S. Howard, and Y. Chen, American Preclinical Services, Minneapolis, MN.

A challenge in developing in vitro alternatives for sensitization testing is addressing multiple events in the adverse outcome pathway. While a number of approaches have been developed within the last ten years, they have mainly been applied only to chemical test articles. The main challenge in translating these tests to medical devices is the heterogeneity of materials present and the number of controls that need to be applied in the extraction process. To address this disparity, we performed a series of studies to investigate the sensitivity and suitability of in vitro sensitization assays in testing medical device extracts. We assessed methods which could investigate three of the key events in the Adverse Outcome Pathway (AOP) describing skin sensitization. The initiating step involves chemical reactions between sensitizers and skin proteins. The Direct Peptide Reactivity Assay (DPRA) measures the depletion of synthetic peptides when exposed to test chemicals by high performance liquid chromatography. We performed experiments to assess the sensitivity of this method compared to the standard in vivo assay, the Guinea Pig Maximization Test (GPMT), and concluded that the test may be unsuitable to measure sensitizers present at the low concentrations typically found in medical device extracts, underscoring the need to further develop in vitro sensitization assays. We examined the next step of the AOP, the activation of keratinocytes. The Keratinosens test method is a recently developed luciferase-reporter based method. We examined the sensitivity of the Keratinosens method by testing titrations of several previously identified sensitizers, and performed feasibility testing to screen for potential positive and negative controls for extraction. We measured the final key step in the AOP, activation of dendritic cells, using the Human Cell Line Activation Test (hCLAT). This flow cytometry platform detects changes in cell surface marker expression. We determined that concentrations of positive control 2,4-dinitrochlorobenzene (DNCB) nearly 10-times lower than those used in the GPMT could induce a significant positive result. Based on this high sensitivity, we also attempted to determine positive and negative controls for extraction for use with the hCLAT. Overall we have evaluated the relative sensitivity of the three assays, GPMT, DPRA and hCLAT.

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### 2948 Extended Applicability Domain for GARD with Vehicles for Medical Device


Allergic contact dermatitis is induced by certain substances, sensitizers, and the symptoms can severely affect quality of life. To prevent individuals from exposure, chemicals and materials must be safety tested. The biological evaluation of Medical Devices includes skin sensitization assessment (ISO 10993-10:2010) and this commonly involves in vivo assays. However, there is a growing public and economic demand for animal-free models. The GARDTM platform is an in vitro state of the art test developed for the assessment of e.g. skin or respiratory sensitizers. The assay is based on a human myeloid cell line, SenzaCell, and depending on the safety hazard to be assessed, tailor-made gene expression panels are analysed. During the development of GARD, DMSO and water were chosen as standard test item solvents. However, the standard for Medical Device extracts (ISO 10993-12:2012) requires both polar and non-polar extraction vehicles; the latter being a challenge for in vitro assays. In this study it is shown that GARDskin is compatible with both. Initially, the compatibility of GARDskin with saline (0.9% NaCl), G-Biosciences and five selected oils, used as extraction vehicles, was assessed. Saline, Super Refined Sesame Oil (CRODA) and Sesame Oil (Acros organics) did not induce cytotoxicity while two other oils, Highly Refined Sesame Oil (CRODA), Super Refined Olive Oil (CRODA) and Sesame Oil (Acros organics) did not induce cytotoxicity while two other oils, Highly Refined Olive Oil (Sigma) and Ph Eur Olive Oil (Sigma), did. Subsequently, the GARDskin prediction signature (GPS) was analysed for the non-cytotoxic extraction vehicles. The GPS result did not predict sensitization for saline and Super Refined Olive Oil, while the results for Super Refined Sesame Oil showed a minor sensitization signal and Sesame Oil resulted in a major signal. The GARDskin prediction signature is a useful method which detects changes in cell surface marker expression. We determined that concentrations of positive control 2,4-dinitrochlorobenzene (DNCB) nearly 10-times lower than those used in the GPMT could induce a significant positive result. Based on this high sensitivity, we also attempted to determine positive and negative controls for extraction for use with the hCLAT. Overall we have evaluated the relative sensitivity of the three assays, GPMT, DPRA and hCLAT.
to detect sensitizing leachables from spiked materials in both polar (saline) and non-polar (Super Refined Olive Oil) extraction vehicles. This broadens the applicability for GARDskin to include Medical Device testing.

**2949 Alternative Tests to Replace Ocular Irritation Animal Test in Medical Device Using Reconstructed Human Corneal Epithelium**

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The alternative methods to ocular irritation animal test in medical devices were evaluated using irritant and non-irritant polymer samples with an in vitro eye irritation test using three dimensionally reconstructed human corneal epithelium (SkinEthic HCE) models produced by EPISKIN. The results were compared with the in vivo rabbit Draize eye irritation test, as well as other alternative test to animal study, the BCOP. Test samples were prepared in polymer form to mimic the state of the medical devices. The 5 irritant polymer samples were examined which were Genapol X-80 in PVC, 15% SDS in silicone, 4% Genapol X-100 in PVC, 25% Heptanoic Acid in silicone and 0.75% ZDEC Polyurethane that contained irritant chemicals in the form of solid polymer. The 3 non-irritant polymers were used which were Silicone Cured Rubber, Elasthane 80A and High Density Polyethylene (HDPE). All the test samples were extracted using polar (saline) and non-polar (sesame oil) vehicles. Extracts of irritant polymer samples were subsequently analysed by ICP-OES and LC-MS. As a result, SkinEthic HCE models yielded a consistent decision for irritant and non-irritant extracts. The results were similar in BCOP, with the exception of non-polar extracts of Genapol X-80 in PVC and polar extracts of 25% Heptanoic Acid in silicone in BCOP, for which no prediction could be made. Also, all the test samples' extracts were non-irritant results in the in vivo test. The analyses of polar extracts of 15% SDS in silicone and 4% Genapol X-100 in PVC showed that the concentration of irritant chemicals were 54.626 mg/l and 196.16 mg/l, respectively. Consequently, these observations suggest that the human corneal epithelium (SkinEthic HCE) could be considered as alternative tests to replace ocular irritation animal test in medical devices.

**2950 Development and Validation of a RHE Model with Impaired Barrier Function to Assess Class IIIb Medical Device Biocompatibility**

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Medical devices (MD) composed of substances belong to a large heterogeneity of products intended by the manufacturer to be used, alone or in combination, for specific human medical purposes and which do not achieve their principal intended action by pharmacological, immunological or metabolic mechanical means. The EU Medical Device Regulation n. 2017/745 (MDR) indicates that the physical and functional properties of the skin barrier are discriminating factors for MD classification: MD applied to intact skin are classified as IIIa while MD that come into contact with injured skin or mucous membranes are IIIb. In order to address the biocompatibility of substance based MD that are intended to come into contact with injured skin (IIIb), a reproducible epidermal injured model with impaired barrier function has been developed by using a commercially available and standardized reconstructed human epidermis model (RHE, EpiSkin Lyon). The injured model has been induced by a mechanical abrasion targeting the epidermal physical barrier, the stratum corneum (SC) and the tight junctions (TJs) associated to granular layer without involving deeper epidermal structures. The model presents an impaired barrier function at the moment of MD application even so in a context of tissue homeostasis. A multiple endpoint analysis (MEA) approach has been adopted to characterize the injured model at 1h and 24h from injury. The results in presence of negative and positive controls (saline solution, Sodium Lauril Sulfate and Lactic Acid) have shown that the cellular viability quantified by MTT was not affected; on the contrary, the injured RHE model has shown an increased permeability to caffeine during the first hour and the impairment of TJs with significant reduction of TEER (Trans-Epithelial-Electrical-Resistance) values. The most interesting result to confirm the impairment of the barrier after 1h and up to the final readout of 24h has been the biotin assay that has shown an increase of biotin quantified by immunofluorescent detection related to the increased barrier permeability also confirmed by H&E staining. The reproducibility of the results has been assessed in n=6 different batches of the RHE model suggesting the interest of the use of this RHE model with impaired barrier function to assess the irritation potential of MD or derma-pharmaceutical products on fragile skin with impaired barrier function. The model seems a robust and predictive tool to assess the biocompatibility of Class IIIb MD taking into account the classification requirements of the MDR.

**2951 Assessment of Thrombogenicity of the CardioGard Gen2 Emboli Protection Cannula in a Fully Anti-coagulated Swine Cardiopulmonary Bypass Model**

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Thrombogenicity testing for blood contacting medical devices is required by ISO 10993-4 for regulatory approval. Issues arise when devices are complex in design; not simple catheters suitable for intravenous (IV) deployment. There is also a desire to assess device thrombogenicity in models that mimic clinical use. We designed an in vivo thrombogenicity assay for a dual lumen aortic cannula, the CardioGard Emboli Protection Cannula-Gen 2, a device specifically designed for use in cardiopulmonary bypass. The device is intended for use only with clinically relevant anticoagulation and is not amenable to IV deployment in either standard in vivo models or in vitro blood loop designs. Differential blood flow rates through the dual lumens of the cannula is a feature in the clinical use of the device and were also deemed critical when simulating clinical use. As a result, we deployed the device in the aorta of three swine on cardiopulmonary bypass for 6 hours with anticoagulation and ACT management and then assessed acute thrombogenicity. After thoracotomy, each animal was placed on cardiopulmonary bypass using the CardioGard as the arterial supply cannula. Once each animal was successfully implanted, the heart was arrested and bypass was maintained for 6 hours with a flow rate of 5 L/min outlet and 1 L/min inlet. Heparin was administered throughout the study to maintain ACT between 400 and 600 seconds. Complete blood counts, and coagulation parameters were also monitored. At the conclusion of the bypass duration, the animals were euthanized and the devices were removed for visual scoring of surface thrombus. Semi-quantitative thrombus assessment was performed by visual inspection of the CardioGard cannula and a lumen of the aorta of ascending aorta. The CardioGard return line filter was also visually assessed for thrombus deposition. All evaluated device surfaces and filters had no formed thrombus and scored “0” for thrombogenicity (no visible surface thrombus deposition). All device surfaces that were visually assessed for thrombus formation had no deposition evident. There was also no evidence of thromboembolism in the brain, kidneys and liver when examined grossly. Overall this study demonstrated the capability of a complex surgical procedure to suitably evaluate the potential thrombogenicity of an extravascular blood contacting device in a manner consistent with clinical practices and compliant with ISO 10993-4 for regulatory review and approval.

**2952 Investigation of Neonatal IPA Exposure from IPA-Filled Caps Used to Disinfect Luer Valves**

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IPA-disinfection cap products are commonly used in neonatal intensive care units (NICUs) and exempted from US regulatory approval. Sauron et al. (2015) reported isopropyl alcohol (IPA) filled caps used to disinfect luer valves can potentially result in excessive IPA exposure (i.e. intravenous) to neonatal patients. Sauron et al. (2015) simulated the worst-case clinical use conditions of commercially available IPA-filled disinfection caps and needle-free luer valves and reported (a) luer valves (normally clear) become opaque after simulated use with IPA-filled disinfection caps and (b) newborns/neonates are at risk of exposure to potentially harmful levels of IPA from the disinfection caps. After review of the general scientific and toxicological merit of the Sauron et al. (2015) report, we concluded the reported level of IPA is not small; however, inadequacies of the reported analytical and toxicological risk assessment methods limits further consideration. We conducted investigations on the impact of IPA-filled disinfection caps on needle-free luer valves and observed (a) luer valves (originally clear) become cloudy within 24-hours of contact with IPA solution or IPA-filled disinfection caps and (b) needle-free luer valve seal integrity became compromised based on an air leak test. These preliminary findings support a potential interaction between the IPA in the IPA-filled disinfection cap and the needle-free luer valve. Further investigation is required to understand potential IPA exposure to neonates in NICUs from the use of commercially available IPA-filled disinfection caps and luer valve combinations. Reference: Sauron et al. (2015) Using isopropyl alcohol impregnated disinfection caps in the neonatal intensive care unit can cause isopropyl alcohol toxicity. Acta Paediatr 104, e489–e493.
2953 Synergistic Toxicity of a Dental Monomer and Nicotine

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Tobacco products as well as nicotine containing replacements contain relatively high levels of nicotine. Research investigating the toxic potential of nicotine has to a large extent focused on interaction with nicotinic cholinergic receptors. Recent studies, however, indicate that nicotine interact with autophagy. The latter could be due to the reported lysosomotropic property of nicotine that may impair lysosome function. The monomer 2-hydroxyethyl methacrylate (HEMA) is one of the major components of many resin-based restorative materials and dental patients are exposed during and after treatment. HEMA has a cytotoxic potential in vitro, and studies suggest that cells adapt to HEMA exposure by increasing the autophagic capacity. The aim of this study was to compare the isolated and combined exposure to HEMA and nicotine on cell viability. As HEMA seems to rely on protection through autophagy and nicotine may impair this pathway, combined exposure could result in synergistic toxicity. A human oral squamous carcinoma cell line PE/CA-PJ49 was exposed to HEMA (0-2 mM) and nicotine (0-10 mM). The cell viability was measured by MTT assay. Western blotting was used to quantify the autophagy related protein p62/SQSTM1 (p62), a protein that is degraded during autophagy. During increased requirement for autophagic flux, p62 synthesis increases. Exposure to nicotine or 2 mM HEMA for 24 h had no measurable effect on cell viability. In contrast, combined exposure to 2 mM HEMA and nicotine reduced the cell viability to less than 50% of control. Cells exposed to nicotine for 16 h showed significant increased p62 level. This level increased further when cells were exposed to both nicotine and 2 mM HEMA. HEMA alone, however, had no measurable effect on the p62 level. In summary, our results show that combined exposure to HEMA and nicotine cause synergistic toxicity. The effect of HEMA and nicotine on p62 further support that this effect is caused by the hypothesized effects on autophagic flux. Increased autophagy caused by HEMA may not affect the level of p62 as both synthesis and degradation increase. Combined exposure to HEMA and nicotine will increase p62 synthesis and block degradation, resulting in higher p62 levels than nicotine alone. Simultaneously, the presence of damaged cellular molecules may explain the increased toxicity. Our results suggest that combined exposure to nicotine and HEMA result in synergistic toxicity and that inhibition of autophagic flux by nicotine is a key event causing this synergy.

2954 Chemical Characterization of Acrylonitrile Butadiene Strene 3D Printed Medical Devices

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Additive manufacturing, otherwise known as 3D printing, research has become exceedingly popular in the recent years. According to a recent Reuters news report, 3D printing has provided a new avenue for medical application-based anatomic surgical models and medical devices. While 3D printing is becoming relevant in medical devices, a variety of factors can affect the biocompatibility of a medical device. Chemical characterization of medical device extractables by mass spectrometric techniques has emerged as a more selective alternative to traditional biocompatibility testing of medical devices, which utilizes biological test systems. We investigated the extractables profile of two specific Acrylonitrile Butadiene Strene (ABS) feedstocks. These feedstocks were then 3D printed into casts. The aim was to determine whether the extractables profile would be altered by 3D printing and thereby potentially affect the biocompatibility. Forearm casts were manufactured from biocompatible Stratasys-M30i and commercial PushPlastic filaments by Fused Filament Fabrication (FFF). Materials tested included the filaments prior to printing, a 3D printed cast for each filament, and a post processed 3D printed cast made from the PushPlastic filament. Sample extraction was performed with 3 different solvents (water, isoproply alcohol and hexane) at 50°C for 24h with gentle agitation. The solvent 7890B GC/MS was used to determine volatile/semi-volatile extractables with NIST 2017 spectral database for compound identification. GC/MS analyses tentatively identified over 60 extractables in isopropanol and hexane extracts of all materials, including extractables such as acetonphenone, and styrene-acrylonitile (SAN) trimers. No water soluble extractables were found by GC/MS. Prints made of M30i filament showed fewer unique identified extractables than the prints made of PushPlastic filament based on the volatile extractables from hexane and isopropanol extractions. Further, the finished PushPlastic 3D print contained fewer identified compounds than the unfinished PushPlastic 3D print. This analysis determined that the printing process utilized herein either introduces new compounds or makes otherwise unextractable analytes available. Ongoing work includes rapid analysis of extractables and leachables by DART-MS, as well as performing a toxicological risk assessment on the identified extractables.

2955 Aerosol Dispersion Measurement of Lung Mixing Volume

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Delivery of aerosolized medication to the lung assumes a mixing volume based on simple measures of inhaled volume. However, using a Chemical Engineering approach for evaluating stirred reactor vessels, it is possible to measure the actual volume involved in the mixing of an aerosol bolus. In theory, the actual volume should inform delivery of aerosolized medication to better calculate dose and, the dose of aerosolized medication should penetrate into the lung to a volume equivalent to the inhaled volume. However, in the case of airway obstruction it is not uncommon to supplement aerosol delivery with Heliox to achieve greater penetration into partially obstructed areas. Thus, a measure of penetration might, additionally, quantitatively inform treatment by providing a measure of penetration. Physician-scheduled pulmonary function testing were offered the opportunity through informed consent to perform aerosol dispersion testing. A patient breathed a $0.7\mu$m corn oil aerosol in a 50 ml bolus to a depth of approximately 400 ml into the patient's lungs. Concentration and volume data were fit to a previously tested sequential series stirred reactor vessel equation. The solution to the equation provided two parameters defined as the equivalent mixing volume and as the mixing coefficient or Peclut number. The ratio of the two parameters can also be used to delineate asthmatic subjects from asymptomatic subjects ($p<0.05$). Asthmatic patients displayed a range of equivalent mixing volume values between 25 and 100% of the one liter tidal volume. Those with the lowest percent predicted values for spirometry as seen in subjects with fixed airflow obstruction, were also the most likely to have lower equivalent mixing volumes. In patients with COPD the equivalent mixing volume appeared to be greater than 150% of that predicted by the actual inhalation volume due to increased airways’ time constants resulting in longer emptying times. Previous work with smoking subjects and current work with patients with COPD and asthma reveal different trends in the change of equivalent mixing volume with worsening symptoms. As a general trend, the equivalent mixing volume appears to increase as obstructive airways’ time constants increase with asthma. Therefore, equivalent mixing volume may also be used to differentiate between asthma and COPD. This indicates that there is a need for methods to evaluate increased penetration of nebulized medication in patients with obstructive airway lung diseases.
Bioabsorbable polymers such as poly(lactic acid), poly(glycolic acid), and poly(lactic-co-glycolic acid) are widely used in medical devices. Although lactic acid and glycolic acid, the final degradation products, are biological compounds found naturally in the human body, high concentrations in the local microenvironment may result in adverse biological effects. The goal of this study was to evaluate the effects of different concentrations of lactic acid and glycolic acid as well as changes in pH on cellular responses and hemocompatibility. Human coronary artery endothelial cells (HCAECs) were exposed to different concentrations of lactic acid and glycolic acid (0.25 mg/mL to 5 mg/mL). Cellular responses were evaluated using the MTT assay (viability), the DCFDA reactive oxygen species assay, and morphological assessment. Hemocompatibility was evaluated using in vitro assays for hemolysis, coagulation cascade activation, and platelet aggregation. After hemolysis testing, polymer degradants were exposed to human whole blood (5 U/mL heparin) diluted to a hemoglobin concentration of 125 mg/dL, with PBS and evaluated using red blood cell counts from an automated cell counter. For coagulation cascade activation, pooled human plasma was treated and assessed using the activated partial thromboplastin time assay. Finally, platelet aggregation and platelet activation were analyzed after exposure of human platelet rich plasma (200K platelets/μL; 1 U/mL heparin) to lactic acid and glycolic acid. Platelet aggregation was analyzed by platelet count using an automated cell counter. For platelet activation, the expression of P-selectin (CD62P) was investigated using flow cytometry. HCAECs exhibited significant cytotoxicity (<70% viability) after exposure to 1.25 mg/mL glycolic acid and 1.75 mg/mL lactic acid. No significant increase in ROS was observed at any concentration. Adverse blood responses including hemolysis, platelet activation, platelet aggregation, and coagulation were also observed after treatment with certain concentrations of lactic acid and glycolic acid. These results suggest that high concentrations of polymer degradants in the local microenvironment may result in adverse biological responses.
2962 Predictive Capacity of Vitrigel-EIT (Eye Irritation Test) Method

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Background: The Vitrigel-EIT method is an in vitro eye irritation assay using a human corneal epithelium model fabricated in a collagen vitrigel membrane (CVM) chamber. Widespread eye irritancy of chemicals is predicted by analyzing the time-dependent changes of trans-epithelial electrical resistance (TEER) values for 3 minutes after exposing chemicals to the culture models. In this study, we aimed to define the applicability domain of the Vitrigel-EIT method by testing a total of 114 chemicals. Also, we estimated the predictive capacity of the test method under the applicability domains. Methods: The eye irritant potential of test chemicals was judged by the Vitrigel-EIT method as irritant or non-irritant, and subsequently the correlation with GHS classification was evaluated. Also, the applicability domain of Vitrigel-EIT method was defined by investigating the physicochemical properties of false-negative chemicals. Results: Discussion: The classification by the Vitrigel-EIT method was in accordance with the GHS categories on 85 test chemicals in the total of 114 chemicals, indicating the accuracy of 74.6%. Meanwhile, eight chemicals were predicted as false-negatives, indicating the false-negative rate of 12.9%. In addition, 21 chemicals were predicted as false-positives, indicating the false-positive rate of 40.4%. Six of the eight false-negative chemicals were acidic, and one of the two non-acidic false-negative chemicals was a water-insoluble solid easily separated from a culture medium. Therefore, we concluded that chemicals with a logP value of 2.5 or more and a density of either less than 0.95 g/cm³ or over 1.10 g/cm³ from the original 114 chemicals. Under the applicability domain, false-negative positive rates were respectively 20.0%, 39.2%, and 79.4%, respectively. These results suggest that the eye irritant potential of chemicals was accurately judged by incorporating the applicability domain into Vitrigel-EIT method.

2963 Implementation of In Vitro Eye Irritation Test Method Using SkinEthic HCE and Between-Laboratory Reproducibility Evaluation in China

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SkinEthicTM reconstructed human corneal epithelium (HCE) in vitro eye irritation test method was adopted by OECD as one of Validated Reference Methods (VRMs). In 2017, following the adoption and commercialization of SkinEthicTM HCE model in China in 2018, the present study was aimed to implement SkinEthicTM (HCE) in vitro eye irritation test method in China according to TG 492. In phase I, 11 chemicals (including one reference chemical from TG 492) as training set was assessed by two operators in each laboratory (Number 1: 100% positive rate and accuracy of 12.3%, Number 2: 20% positive rate and 30% accuracy). In phase II, remaining 14 reference chemicals from TG 492) as training set was assessed by two operators in each laboratory. In phase I, 11 chemicals performed by two operators in each laboratory obtained high within-lab reproducibility, showing Number 1: 100% sensitivity and 86.5% (36.3/42) accuracy. In conclusion, SkinEthicTM HCE in vitro eye irritation test method was successfully transferred in China using the model produced in this country, showing high predictivity and good within-lab reproducibility. The current study provides solid scientific and technical foundation to enable the implementation of in vitro eye irritation test, also further supports and promotes application of alternative methods in China.

2964 Comparing Therapies to Reduce Ocular Sulfur Mustard-Induced Injuries

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Sulfur mustard, a chemical weapon used in World War I, the Iraq-Iran War, and in the 2015 Syrian conflict, is a vesicant that injures the eyes, lungs and skin. Ocular exposure leads to corneal microblistering, ulceration, and possibly loss of the corneal epithelium from its stroma, neovascularization, scarring, and a long term possibility of developing dry eye disease. Separation of the corneal epithelium from its stroma can cause a loss of vision. No drugs are as yet US FDA approved for ocular mustard injury. Here two US FDA-approved drugs for other ocular injuries, oxetertacycline and Restasis, were tested as therapies for ocular mustard injury. Used on unjured corneal organ cultures these drugs showed no adverse effects. Other corneal organ cultures were exposed to nitrogen mustard (NM) for 2 hr, followed by recovery for 22 hr. During the 22 hr recovery, some corneas received no therapy, others received Restasis (one drop every 12 hr), and others received oxetertacycline treatments 3 times in 24 hr by preparing a solution where 40 µL delivered 0.2 mg oxetertacycline. After the exposures and treatments, corneas were collected and sectioned to examine the histology of the microscopic injury. The images with either drug showed a reduction in NM-induced ocular damage compared to eyes receiving no drug after exposure, as assessed by the degree of epithelial-stromal separation. These data supported further testing of the therapies on in vivo rabbit ocular organ cultures using sulfur mustard (SM). The same ocular tests were performed at 24 hr after a 2 hr SM exposure. Restasis was applied every 12 hours, as was done in the organ cultures, but the in vivo oxetertacycline experiment included only 2 applications per day rather than 3. Both the histology of organ cultured corneas and in vivo exposed corneas indicated that Restasis was more effective for attenuating ocular injury from NM and SM. Future work includes adding an antiangiogenic to Restasis to see if this reduces neovascularization.

2965 Regulators of Toxicant-Induced Cornea Epithelial Injury Identified by High-Throughput siRNA Screening


Some toxicant exposures to the eye produce a transient injury, while others produce delayed and progressive corneal injury which compromises vision. These injuries often share similar clinical features such as corneal ulceration, opacity, and neovascularization. Studies have shown that there may be some common cellular and molecular mechanisms that drive these delayed progressive clinical features. Although some of these mechanisms have been described, they are not fully understood even for high risk ocular toxicants. In cases where there are common mechanisms for delayed injuries, chemicals with different toxicological properties are likely to elicit different molecular responses early after exposure. It is important to understand these responses as they may present opportunities for early intervention. We have performed siRNA high throughput screening (HTS) in an effort to understand the molecular mechanisms of toxicant-induced corneal injury. We selected hydrogen fluoride (HF) and chloropicrin (CP) for study by cross-referencing Chemical Terrorism Risk Assessment list agents with the ToxNet reports of industrial accidents to find chemicals that present the greatest risk of ocular injury via vapor exposure. Two immortalized human corneal epithelial cell lines were used for study: SV40 immortalized corneal epithelial cells and telomerase immortalized corneal epithelial cells. Microarray data from HF- and CP-injured mouse corneas were mined and used to select 3120 targets from preconfigured Dracmacon Human Drugable siGENOME SMARTpool libraries. The endpoints assessed for HTS were IL-8 levels in cell culture medium and cell viability. Targets were down-selected into validation studies which utilized phenotype restoration and endpoints such as multiplex cytokine analysis and high content analysis to understand toxicant effect and target function. The list of validated siRNA targets that regulate HF- and CP-exposed cell viability and IL-8 production includes molecules such as transcriptional regulators, kinases, phosphatases, solute carriers, and metabolic proteins just to name a few. However, our results show that there is little to no similarity in the molecular responses to these two toxicants. These findings suggest that some early interventions for toxicant ocular injury may need to be toxicant-specific.
Distinctive Roles of NURR1 in Modulating Inflammation, Autophagy and Cell Migration in Human Retinal Pigment Epithelial Cells

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Age-related macular degeneration (AMD) is the leading cause of vision loss in the elderly, occurring due to degeneration and/or dysfunction of the retina and supporting retinal pigment epithelium (RPE). Though multi-factorial, in which genetic and environmental factors play a role, epidemiological studies indicate that the two established risk factors for AMD are aging and cigarette smoking. Phenotypically, it is characterized by accumulation of lipid- and protein-rich extracellular deposits below the RPE and degeneration of the photoreceptors. These degenerative changes are thought to be due to a lack of autophagy, a process of recycling intracellular components, and 50% of patients with neovascular AMD are treated with anti-vascular endothelial growth factor (VEGF) agents. Therefore, therapies that could induce autophagy as a means to clear toxic deposits and protect RPE cells may be of therapeutic benefit.

NURR1 is a nuclear receptor that is a transcription factor and has a crucial role in maintaining the function of the RPE, which is a single layer of cells that serves as the barrier between blood vessels and the photoreceptors in the retina. NURR1 is known to regulate the expression of several genes involved in autophagy and is recently studied to be a potential therapeutic target for AMD.

In this study, we characterized the role of NURR1 in autophagy and cell migration in human RPE cells. Using a novel small molecule CSE, we observed a dose-dependent increase in intracellular NURR1 expression and autophagy, as measured by increased LC3-II expression and increased RPE cell migration in human RPE cells. These observations suggest that NURR1 may be a viable target to improve the health of RPE cells. In support of this, we found that over-expression and/or ligand activation of NURR1 increased intracellular expression of pro-inflammatory cytokines including interleukin-6 and monocyte chemotactic protein-1 in normal human RPE cells, regardless of age. CSE induced NURR1 expression and activity in human RPE cells as evidenced by qPCR, western blots and NURR1-luciferase reporter activity assays. This activation was more pronounced in RPE cells isolated from aged donors, implicating that aged RPE cells expressing less NURR1 are more susceptible to environmental stimuli. CSE also enhanced LC3-II expression in human RPE cells, indicative of induced autophagy. Ligand activation of NURR1 modulated LC3-II expression in human RPE cells, suggesting the involvement of NURR1 in autophagy. Finally, while ligand activation of NURR1 inhibited cell migration in human RPE cells as assessed in wound-healing assays, over-expression of NURR1 attenuated TNFα-induced migration in human RPE cells. Combined, these novel observations demonstrate that the relative expression level of NURR1 is critical for the autophagic and migratory functions of human RPE cells. The inhibitory effect of activating or over-expressing NURR1 on modulating migration in human RPE cells implicates a potential therapeutic application for AMD patients.

Ocular Toxicity Associated with a Structurally Related PI3Kβ Selective Inhibitor

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The toxicology profile of a novel candidate phosphoinositide-3 kinase beta (PI3Kβ) selective inhibitor, C-377C, was evaluated in a 7 day repeat dose dog study. In addition to routine ophthalmic exams, detailed ocular assessments of electroretinography (ERG) and optical coherence tomography (OCT) were included based on the microscopic finding of retinal degeneration observed in a previous study with a structurally related compound. Male beagle dogs (3/group) were dosed daily with C-377C at 0 (vehicle), 10, 40 and 100 mg/kg/day. Based on poor tolerability, high dose animals were euthanized after the second dose on Day 2. Dosing was suspended in the 40 mg/kg/day group on Day 4, and resumed on Day 5 at 30 mg/kg/day. Ocular toxicity, characterized by both structural and functional effects, was present at the low and mid dose levels and was considered likely irreversible. Clinical observations indicated decreased response to visual stimuli, dilated pupils, red conjunctiva and ocular discharge in all animals beginning on Day 4 through the end of the study. Ophthalmic evaluations, ERG and OCT were conducted pre-dose and on Day 6. ERG assessment following dark-adaptation indicated that all dogs in the 10 and 40/30 mg/kg/day groups had no response to single light flashes of 0.01, 0.06, or 2.5cd/s.m-2. Ophthalmic evaluation (slit lamp biomicroscopy/indirect ophthalmoscopy) revealed decreased tapetum in 2/3 dogs at 10 mg/kg/day with complete loss of tapetum in 3/3 dogs and retinal detachment in 2/3 dogs at 40/30 mg/kg/day. OCT imaging confirmed the tapetal changes and retinal detachment. Additional effects noted with OCT included photoreceptor layers appearing as an indistinct single hyper-reflective band and swelling of the optic nerve in the 10 and 40/30 mg/kg/day administration group. No change was reported to be synchronized with circadian rhythms and peak at 2 hr after light onset. In our previous experiment, UNC569, a specific MerTK inhibitor, induced morphological changes in the RPE and photoreceptor cells in the retina in mice. To investigate the influence of dosing time on UNC569-induced retinal toxicity, UNC569 at 100 mg/kg was orally administrated to male mice at Zeitgeber time (ZT; the time of the biological clock in 12/12-hour light-dark cycle where the start time of the light and dark cycle is ZT 0 and ZT12, respectively) 5.5 or ZT22 for 28 days. Electron microscopy was conducted at ZT22 after the final dosing. Additionally, the visual cycle components (11-cis-retinol, 11-cis-retinal, all-trans-retinal, and all-trans-retinol) which play an important role in maintaining retinal homeostasis were quantified by LC/MS/MS. As results, ultrastructurally, the number of phagosomes and phagolysosomes in the RPE increased in both ZT5.5 and ZT22 administration groups, while endoplasmic reticulum dilatation of RPE and chromatin aggregation of photoreceptor nuclei were observed in the retina administered with the drugs. This study was performed in all the components of the visual cycle, indicating that the involvement of the visual cycle on the changes might be low. The present result suggested that the lesion was deteriorated by the absence of physiological MerTK phosphorylation started at ZT2, resulting in severer changes in the ZT22 group.

Evaluation of AAV-Based Gene Delivery to Retinal Cells in Histological Sections in Animal Toxicity Studies

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Gene transfer with adeno-associated virus (AAV) vectors is extensively used for retinal gene therapy applications in preclinical studies and clinical trials due to prolonged expression, low immunogenicity, and established record of safety and efficacy. To allow the direct visualization of vector trafficking and protein localization in transduced cells, green fluorescent protein (GFP) is widely used as a gene expression reporter and a molecular and cellular tag. Previously we have demonstrated the efficacy of multiple methods of GFP detection in histological sections. The purpose of this study was to investigate and establish reliable and reproducible methods for evaluation of GFP expression and to analyze AAV trafficking through tissue biodistribution of gene transduction, protein synthesis and secretion, in an experimental ocular toxicity study. Female C57BL/6NTac mice were administered a commercially available AAV vector containing a gene for GFP (AAV2-GFP) via subretinal injection in the eye. Group 2 animals also received anti-inflammatory therapy to investigate effects of ocular inflammation on the efficiency of vector transduction in different retinal cell types. Animals were euthanized 43 days following injection. Histological sections of formalin-fixed paraffin-embedded eyes were examined microscopically. Detection of GFP reporter was performed on serial sections by immunohistochemistry (IHC) and RNA in situ hybridization (ISH). IHC and ISH staining were evaluated using H-score and a modified RNAscore® scoring system respectively. Commercially available tissue and cell lines stably expressing GFP were used as controls and to evaluate the specificity and sensitivity of the methods. Microscopic findings in both groups were attributed to the subretinal dosing procedure. IHC for GFP revealed negative to minimal labeling in inner and outer nuclear layers and minimal to moderate labeling in the photoreceptor (PHR) layer. In retinal pigment epithelium (RPE), labeling usually occurred in groups of approximately 2-10 adjacent cells. Similar findings were noted with RNA ISH. Statistical analysis of semi-quantitative IHC and ISH found no significant differences between groups 1 and 2, although there was a slight apparent increase in IHC GFP labeling and in the number of transduced cells detected by ISH in the PHR and RPE in group 2 in comparison to group 1. Our study demonstrates approaches for evaluation of IHC and ISH by semi-quantitative scoring systems that allow interpretation and reporting of results in toxicity studies.
Arsenic is wide spread in the environment and is a class I human carcinogen. Chronic arsenic exposure causes lung, skin, and bladder cancer. Paradoxically, arsenic is a potent chemotherapeutic against hematological and solid tumors, and can potentiate the cytotoxic effects when used in combination with other DNA damaging chemotherapeutics, such as hyperthermia and cisplatin. Approved by US FDA, arsenic is a therapeutic for treating acute promyelocytic leukemia (APL). Unfortunately, the use of arsenic is limited among APL patients because up to half experience respiratory events early in the course of the disease, including potentially fatal acute lung injury (aka acute respiratory distress syndrome). Surfactant protein B (SFTPB) plays a critical role in maintaining proper alveolar surface tension and the functionality of healthy lungs, and its absence leads to alveolar collapse that can progress to acute lung injury. SFTPB expression is regulated by Nk2 homeobox 1 transcription factor (Nkx2-1, aka thyroid transcription factor 1, TTF1). In mice, Sftpb transcription is also regulated by SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 (SMARCA, aka BRG1) a chromatin remodeling complex protein that interacts with Nkx2-1. In this study, we demonstrate that arsenic decreases SFTPB transcripts in vivo and in vitro, which can be rescued by dexamethasone. In human alveolar epithelial cells transfected with SFTPB promoter-reporter, arsenic decreased human SFTPB promoter activity. Arsenic also enhances the methylation status of Nkx2-1, thus the transcript level is reduced. A methylation inhibitor, 5-aza-2’-deoxycytidine, rescues the reduction of SFTPB gene promoter activity and SFTPB transcripts caused by arsenic. In summary, we determine that arsenic impairs alveolar surfactant protein B biosynthesis, in part, through Nkx2-1 hypermethylation. These findings could be useful in reducing adverse pulmonary events during arsenic chemotherapy.

How an epigenome’s sensitivity to chemical exposure changes with age is not fully understood. This is particularly significant for the germline where the genome is kept relatively silent through repressive epigenetic marks such as H3K9me3 (histone 3 lysine 9 trimethylation) and H3K27me3 (histone 3 lysine 27 trimethylation). To investigate the relationship between age and epigenetic sensitivity we first used immunofluorescence to quantify changes in the levels of H3K9me3 and H3K27me3 with age in C. elegans germlines. C. elegans are a useful model organism for studying aging because they have a short generation time, are transparent which enables microscopy, and share many canonical age-related pathways with humans such as the insulin-like growth factor signaling pathway. We age-synchronized worms and performed immunofluorescence for H3K27me3 and H3K9me3 at days 1, 3, 5, and 7 of adulthood. We performed 5-7 replicates and for each replicate we quantified the fluorescence intensity of nuclei in 3-4 gonads (10 mid-late pachytene nuclei per gonad). We averaged nuclei area and nuclei intensity for each gonad as well as for all gonads on the same slide. The mean nuclei area decreases with age, the normalized H3K9me3 intensity decreases slightly with age, though the trend becomes weaker at day 7, and variation in H3K27me3 intensity between gonads on the same slide increases with age. The decrease in H3K9me3 intensity could be correlated with the degradation of the nuclear periphery and loss of heterochromatin that occurs with age (Sen et al., 2016). The increased variation in H3K27me3 intensity could be correlated with the increased transcriptional noise and cryptic transcription seen with age (Sen et al., 2015). Next, we will expose worms to the global histone methylation inhibitor 3-deaxaneplanocin A (DZNep). Comparing H3K27me3 and H3K9me3 intensities of the DZNep-exposed gonads to the water-exposed gonads will allow us to investigate changes in epigenetic sensitivity with age. A deeper understanding of how epigenetic sensitivity changes with age will allow us to more effectively test chemicals before they are used in consumer products.

Arsenic pollution in drinking water is a serious public health problem in the world, which affects approximately 150 million people in over 70 countries. People in the affected areas are exposed to arsenic through ingestion of contaminated drinking water and diet on a daily basis. Many human diseases, including neurodegenerative disorders, are engendered by the malfunctioning of proteins vital for important biological processes elicited by protein misfolding. We or in protein quality control during translation. Over the last two decades, it has been proposed that arsenic could affect protein folding and translation, resulting in the proteotoxic stress. Several epidemiological studies have revealed that higher incidence rate of neurodegenerative disorders in a population is correlated with chronic arsenic poisoning. However, the detailed molecular mechanism underlying the arsenic-induced proteotoxic stress is largely unknown. Here, we propose that arsenic could lead to proteotoxic stress through impairing the cotranslational protein quality control pathway by downregulating the E3 ubiquitin ligase activity of ZNF598. We showed that arsenic could interact with ZNF598 in cells, and arsenite could substitute the zinc ions bound with the RING finger domain of the protein through competitive binding. Additionally, our LC-MS/MS-based proteomic data revealed that a 24-hour exposure of 5 μM NaAsO\text{2} resulted in the decrease of specific lysine residues on the specific ribosomal proteins (i.e. 138/139 in RPS10 and K4/8 in RPS20) which are mainly ubiquitylated by the E3 ubiquitin ligase ZNF598. Together, our results support that arsenite could bind to the RING finger domain of ZNF598 by competing zinc ions, thereby impairing the regulatory ubiquitination on RPS10/20 and compromising the co-translational protein quality control pathway. Our study suggests a novel mechanism underlying the arsenic-induced proteotoxic stress in human cells.

How an epigenome’s sensitivity to chemical exposure changes with age is not fully understood. This is particularly significant for the germline where the genome is kept relatively silent through repressive epigenetic marks such as H3K9me3 (histone 3 lysine 9 trimethylation) and H3K27me3 (histone 3 lysine 27 trimethylation). To investigate the relationship between age and epigenetic sensitivity we first used immunofluorescence to quantify changes in the levels of H3K9me3 and H3K27me3 with age in C. elegans germlines. C. elegans are a useful model organism for studying aging because they have a short generation time, are transparent which enables microscopy, and share many canonical age-related pathways with humans such as the insulin-like growth factor signaling pathway. We age-synchronized worms and performed immunofluorescence for H3K27me3 and H3K9me3 at days 1, 3, 5, and 7 of adulthood. We performed 5-7 replicates and for each replicate we quantified the fluorescence intensity of nuclei in 3-4 gonads (10 mid-late pachytene nuclei per gonad). We averaged nuclei area and nuclei intensity for each gonad as well as for all gonads on the same slide. The mean nuclei area decreases with age, the normalized H3K9me3 intensity decreases slightly with age, though the trend becomes weaker at day 7, and variation in H3K27me3 intensity between gonads on the same slide increases with age. The decrease in H3K9me3 intensity could be correlated with the degradation of the nuclear periphery and loss of heterochromatin that occurs with age (Sen et al., 2016). The increased variation in H3K27me3 intensity could be correlated with the increased transcriptional noise and cryptic transcription seen with age (Sen et al., 2015). Next, we will expose worms to the global histone methylation inhibitor 3-deaxaneplanocin A (DZNep). Comparing H3K27me3 and H3K9me3 intensities of the DZNep-exposed gonads to the water-exposed gonads will allow us to investigate changes in epigenetic sensitivity with age. A deeper understanding of how epigenetic sensitivity changes with age will allow us to more effectively test chemicals before they are used in consumer products.
2974 Effect of Smoking and Air Pollution on Peripheral Blood RNA Modifications in the Beijing Truck Driver Air Pollution Study

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Post-transcriptional modifications of RNA play a fundamental role in regulation of the human transcriptome. The so-called epitranscriptome is critical for health and disease, and can be modulated by cellular stress. However, effects of environmental toxicants on the epitranscriptome remain unclear. Our objective was to examine associations between tobacco smoking and air pollution with 10 RNA modifications in whole blood. Using the Beijing Truck Driver Air Pollution Study, we investigated 10 total RNA modifications in peripheral blood collected from 111 subjects in Beijing, China in 2008. We measured 1-methyladenosine (m1A), N6-methyladenosine (m6A), 2'-O-methyladenosine (Am), N6,2'-O-dimethyladenosine (m2Am), 3'-methylcytidine (m3C), 2'-O-methylcytidine (Cm), N6-acetylcysteine (acC), pseudouridine (Ψ), 5'-methyluridine (m5U), and 2'-O-methyluridine (Um) with nLC MS5. We examined associations between smoking variables (ever/never smoker, pack years (none<3.8 years/3.8 years), environmental tobacco smoke (light/heavy), and smoking on day of visit (present/absent)) and personal air pollution exposure (black carbon (BC) and PM2.5 as measured with a portable monitor over 8 hours) with each modification using linear models. Ever/never smoking and pack years were significantly associated with a 13% decrease in m6A methylation (95% CI: -22.2, -5.6). Smoking on the day of the visit was significantly associated with a 17%-59% increase in m1A, m3C, and m2Am, while m3C, m4C, and m5C were inversely associated with short-term smoking, suggesting a more transient effect. Furthermore, BC was positively associated with m3A. These results provide early evidence into the impacts of environmental toxicants on RNA modifications.

2975 Comparative Analyses of MicroRNA Profiles of E-cigarette Vapour Condensate and Cigarette Smoke Extract-Challenged A549 Cells

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Since their introduction in the United States (US) markets, electronic cigarettes (E-cigs) have become extremely popular amongst people of all ages. As of 2012, 1.76 million students in grades 6 to 12 have been reported e-cigarette users which marked a significant increase in US adolescents. While there is a large body of literature suggesting the risks associated with cigarette smoking, not much information is available regarding effects of e-cigarettes. Considering this, a detailed analysis of the microRNA profiles on cigarette smoke and e-cigarette vapour exposure could uncover essential details regarding the affected genes and regulatory pathways. MicroRNAs (miR) are small RNA molecules that function to regulate gene expression post-transcriptionally. There is evidence of dysregulation of miR expression on cigarette smoke exposure. Similarly, transcriptome profiling of human bronchial epithelial cells exposed to-tobacco und e-cigarette smoke using commercially available miRNA PCR Array (Inflammation and Autoimmunity). Our results demonstrate downregulation of 12 miRs; while upregulation of 3 miRs in TF-ECVC challenged A549 cells. Contrarily, 8 miRs were found to be downregulated on CSE challenge. Two miRs, i.e. hsa-miR-181b-5p and hsa-miR-29a-3p, showed significant fold variation on challenge with TF-ECVC. The hsa-miR-181b-5p has been shown to positively regulate p38 MAPK cascade, while hsa-miR-29a-3p negatively regulates proliferation and apoptosis. Our investigations, thus far reveal that TF-ECVC and CSE exposure impacts a distinct set of miRNA populations which may be responsible for the differences in the nature and intensity of inflammatory responses following the exposures. Support: LBRN Summer Award; Young Clinical Scientist Award (FMARRI-123253, YCSA, Faculty); NIH R15 (7 R15 ES023151 02) and Southern University Foundation Grant (FY17-COSC0015; FY18-020).

2976 Low-Dose Developmental Dieldrin Exposure Alters DNA Methylation at Genes Related to Parkinson’s Disease in the Mouse Midbrain

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Human and animal studies have shown that exposure to the organochlorine pesticide dieldrin is associated with increased risk of Parkinson’s disease (PD). Despite previous work showing a link between developmental dieldrin exposure and increased neuronal susceptibility to MPTP toxicity in male C57BL/6 mice, the mechanism mediating this effect has not been identified. Here, we tested the hypothesis that developmental exposure to low-dose dieldrin increases neuronal DNA methylation via genome-wide changes in DNA methylation. Starting at 8 weeks of age, female C57BL/6 mice were exposed to 0.3 mg/kg dieldrin by feeding every 3 days for 30 days prior to mating; maternal dieldrin exposure then continued for the duration of gestation and lactation until weeks of age, pups were sacrificed and brains were dissected. Offspring DNA was then isolated and dieldrin-related changes in DNA methylation were measured via reduced representation bisulfite sequencing (RRBS). We identified a number of significant differentially methylated CpG sites (DMCs) and differentially methylated regions (DMRs) by developmental dieldrin exposure (FDR<0.05). Furthermore, stratification by sex showed that dieldrin exposure had distinct effects on the male and female epigenome. In the male mice, we found dieldrin-related differential methylation within the gene body of the imprinted Grb10 locus. Grb10 encodes an adaptor protein that interacts with Grb10-interacting GYF Protein 2 (GIYF2), a protein encoded in the PARK11 locus that may play a role in PD-related neurodegeneration. Meanwhile, in the female mice, gene ontology pathway analysis showed an enrichment for differential methylation at genes related to central nervous system development. These genes clustered together in an interaction network, and one of the identified loci - Nr4a2 - encodes a transcription factor that has previously been implicated in PD pathogenesis and development of motor dysfunction and motor phenotypic phenotype. In targeted RNA-sequencing analyses, a protein-coding Nr4a2 transcript showed marginally significant differential expression by dieldrin exposure in female mice (p=0.06). Combined, these data suggest that developmental dieldrin exposure can establish a poised epigenetic state at genes related to PD, and that sex-specific epigenetic changes at these loci may contribute to the development of neurodegenerative disease.

2977 Persistent DNA Methylation Linked to Latent Carcinogenesis after Short-Term Chemical Exposure in Mice

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Early-life environmental factors can influence later-life susceptibility to cancer. Previously, we showed that exposure to dichloroacetic acid (DCA) increased liver cancer in mice 84 weeks after continuous or early life exposures similarly. Mechanistic findings did not support direct cytotoxic, mitogenic, or genotoxic modes of action. Here, we proposed that prior DCA exposure mediates carcinogenic activity through persistent epigenetic effects. Gene expression was measured by targeted RNA sequencing analyses, a protein-coding Ntr4a2 transcript showed marginally significant differential expression by dieldrin exposure in female mice (p=0.06). Combined, these data suggest that developmental dieldrin exposure can establish a poised epigenetic state at genes related to PD, and these sex-specific epigenetic changes at these loci may contribute to the development of neurodegenerative disease.
DCA exposure. Any direct influence of methylation changes on gene expression or specific oncogenic pathways likely occurred during the lifespan of the mouse. This abstract does not represent US EPA policy.

**2978** An Obesity-Associated Gut Microbiome Reprograms the Intestinal Epigenome and Leads to Altered Colonic Gene Expression

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The gut microbiome, a key constituent of the colonic environment, has been implicated as an important modulator of human health. The eukaryotic epigenome is postulated to respond to environmental stimuli through alterations in chromatin features and, ultimately, gene expression. How the host mediates epigenomic responses to gut microbiota is an emerging area of interest. Here, we profile the gut microbiome and chromatin characteristics in colon epithelium from mice fed either an obesogenic or control diet, followed by an analysis of the resultant changes in gene expression. The obesogenic diet shapes the microbiome prior to the development of obesity, leading to altered bacterial metabolite production which predisposes the host to obesity. This microbiota-diet interaction leads to changes in histone modification at active enhancers that are enriched for binding sites for signal-responsive transcription factors. These alterations of histone methylation and acetylation are associated with signaling pathways integral to the development of colon cancer. The transplantation of obesogenic diet-conditioned microbiota into germ free mice, combined with an obesogenic diet, recapitulates the features of the long-term diet regimen. The diet/microbiome-dependent changes are reflected in both the composition of the recipient animals' microbiome as well as in the set of transcription factor motifs identified at diet-influenced enhancers. These findings suggest that the gut microbiome, under specific dietary exposures, stimulates a reprogramming of the enhancer landscape in the colon, with downstream effects on transcription factors. These chromatin changes may be associated with those seen during colon cancer development.

**2979** Epigenetic Effects of Cannabinoids on Bronchial Epithelial Cells

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Cannabis sativa, commonly known as Marijuana, is one of the most commonly used illicit drugs in the USA, with a reported estimate of 6% of the population aged 26 and older having used marijuana in 2015, for a total of 22.2 million users overall. Therefore, the question of the impact of its use on human health becomes critical. Since inhalation is a major route of exposure, the effect of cannabinoids on bronchial epithelial cells also becomes relevant. The current study investigated the epigenetic effects of chronic cannabinoid exposure as well as the set of transcription factor motifs identified at diet-influenced enhancers. These findings suggest that the gut microbiome, under specific dietary exposures, stimulates a reprogramming of the enhancer landscape in the colon, with downstream effects on transcription factors. These chromatin changes may be associated with those seen during colon cancer development.

**2980** Placental Metals and Expression of miRNAs That Target TGF-β Pathway

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Pre-eclampsia (PE) is hypothesized to arise from impaired trophoblast invasion into the maternal decidua, giving rise to shallow placentation. In vitro, trophoblast invasion is impaired when exposed to toxic metals. This effect may be mediated by metals-induced changes in microRNA (miRNA) expression. Specifically, miRNAs that target the transforming growth factor beta (TGF-β) pathway have been previously implicated in PE development. We hypothesized that placental toxic metal levels are associated with the expression of miRNAs that target the TGF-β pathway and that these associations may be modified by placental essential metals. Placenta samples were collected from participants in a case-control study of 172 women participating in the Maternal Oral Therapy to Reduce Obstetric Risk (MOTOR) cohort. We quantified placental metals (arsenic [As], chromium [Cr], Cd, lead [Pb], mercury [Hg], selenium [Se], and zinc [Zn]) using inductively coupled plasma-mass spectrometry, and miRNA expression (miR-26a, miR-101, miR-193b, and miR-199b-5p) using a qPCRs-based assay. The association between individual metals and miRNA expression was examined using multivariable linear regression models. Interactions between toxic and essential metals were assessed using interaction-based models. After accounting for potential confounders, placental As and Cr levels were positively associated with the miR-26a, miR-101, and miR-1990-5p expression, and negatively associated with miR-193b expression. There were no associations between placental Cd, Pb, or Hg levels and miRNA expression. Placental Se and Zn levels were associated with lower miR-193b expression and placental Se was positively associated with miR-101 expression. We also identified significant antagonistic interactions between placental Se and several toxic metals, including As, Pb, and Hg, with respect to miR-26a and miR-193b expression. Placental metal levels are associated with the expression of TGF-β-targeting miRNA. Antagonistic interactions between placental Se and toxic metals indicate that lower placental Se may increase susceptibility to metals-associated changes in miRNA expression.

**2981** Comparison of Mouse Liver and Blood DNA Methylation after Gestational Exposure to Lead


DNA methylation is a key epigenetic mechanism linking early developmental environment to long-term health outcomes. Human population-based epigenetic studies are most often limited to easily obtainable, surrogate sources of DNA, including blood. However, the extent to which toxicant-induced changes in DNA methylation in surrogate tissues mirror those in the target tissues is unclear. The Toxicant Exposures and Responses by Genomic and Epigenomic Regulators of Transcription (TaRGET II) consortium was established by the National Institute of Environmental Health Sciences to address the utility of surrogate tissues as proxies for toxicant-induced epigenetic changes in target tissues. In this study, we compared the methylation of liver and blood in 5-month-old male (N=6) and female (N=6) mice that were perinatally exposed to lead. Experiments were performed using genetically invariant mice 93% identical to C57BL/6J. Two weeks prior to mating, dams were assigned to control or lead-acetate (32ppm) water. To assess DNA methylation, we used enhanced reduced-representation bisulfite sequencing. Differentially methylated regions (DMRs) were identified with an FDR cutoff <0.05 and a methylation difference >10%. Although lead exposure ceased at 3 weeks of age, this analysis revealed thousands of stably modified, sex-specific DMRs in the adult blood (n=1535 and n=1404) and liver (n=1740 and n=3342) of exposed females and males, respectively. Overall, 3 DMRs overlapped between liver and blood in females, and 6 in males. The direction of methylation change with exposure was not concordant between blood and liver at all sites; however, in males, Grfn was hypomethylated, and Mirt135 and Plekhg3 were hypermethylated in both liver and blood of exposed mice. Together, these data demonstrate that perinatal exposure to lead induces sex-specific changes in DNA methylation that persist into adulthood, some of which are consistent across target and surrogate tissues. Ongoing studies in TaRGET II will provide additional exposure-specific insights, and include other epigenetic marks that will enable further refinement of the design and analysis of human studies where target tissues are inaccessible.
2982 Whole Genome Assessment Reveals Altered Methylation in DNA Methylation of Polycomb Repressive Complex 2 Components in Effector/Memory CD4+ T Cells after Continuous and Discontinuous Developmental Exposure to Trichloroethylene

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Trichloroethylene (TCE) is an industrial solvent and water pollutant that promotes autoimmunity/hypersensitivity in both humans and rodents. In auto-immune-prone female MRL +/- mice, adult-only exposure to occupational-level-relevant levels of TCE from the drinking water (500 μg/ml) promoted changes in DNA methylation in regulatory components of the polycomb repressive complex 2 (PC2) in effector/memory CD4+ T cells. These DNA regions bind EZH2, Mtf2, and Jarid2 that are important in CD4+ T cell differentiation and activation, and may regulate autoimmune disease. Developmental exposure to the same dose of TCE during gestation, lactation and early life until postnatal day (PND) 154 generated autoimmunity at PND 259. Since removal of TCE from the drinking water did not prevent disease, we predicted that alterations in DNA methylation of PC2 components would be maintained when TCE was removed from the drinking water. In the current study, whole genome reduced representation bisulfite sequencing (RRBS) was used to compare methylation changes in the CD4+ T cells with noncoding RNA sequences. Results suggest that PC2 methylation in regulatory elements altered by TCE exposure during gestation and lactation could impact methylation in noncoding RNA expression and chromatin modifications. In this study, we characterized the effect of pre-conceptional exposure to PC2 methylations, a persistent organic pollutant, on gene expression to help determine if methylation alterations are promoting transcriptomic alterations, as well as investigate if these findings are tissue-specific or persistent later in life.

2983 Epigenetic Effects of Environmental Chemicals: Integrative Effects on DNA Methylation and Noncoding RNAs

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Epigenetic mechanisms such as DNA methylation, chromatin modifications and noncoding RNAs together comprise the “cellular epigenome” and these mechanisms play an important role in the regulation of gene expression. Environmental factors such as diet, psychological stress and chemical exposure have been shown to modify the epigenome particularly during sensitive windows of development such as embryogenesis and gametogenesis. Some of these effects have been shown to be transmitted to subsequent generations. Most of the previous studies have investigated the influence of environmental factors on epigenetic mechanisms in cell lines. Emerging evidence suggests that there is a role for DNA methylation, noncoding RNAs and chromatin modifications in the development of complex diseases, including neurological system processes, chemical stimuli in senescence, and immune response. From the CD3+/CD8+ T cells, we were able to further gate the cells into CD3+/CD8+/CD16+, CD3+/CD8+/CD56+, CD3-/CD8+ and CD3-/CD8-. Interestingly, only the CD3+/CD8+ populations showed a significant increase (3.97%; p = 0.016) in smokers. Previous studies have demonstrated a role for Gm1 and Prf1 producing CD8+ T cells in the development of atherosclerotic plaques in mice. Taken together, this increased in latent effector CD8+ (CD3+/CD8+/CD16+) T cells might represent an intermediate stage in smoking-induced atherosclerosis.

2984 Using Mass Cytometry to Identify Leukocyte Population Changes from Cigarette Smoke Exposure

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Exposure to cigarette smoke has been found to impact immune response, leukocyte subtypes, DNA methylation, and expression from human whole blood. To identify cell populations affected by smoking and possibly connect observed immune cell changes with smoking-associated diseases, we characterized gene expression profiles and cell surface marker phenotypes from primary peripheral blood mononuclear cells (PBMCs) from 5 nonsmokers and 5 smokers by single cell RNA sequencing (scRNA-seq) and mass cytometry (CyTOF). scRNA-seq identified a population of Natural Killer (NK)-like CD8+ T cells in smokers which shared gene expression profiles with both NK cells and CD8+ T cells including an increase in FCGR3A (CD16), GZMB (Granzyme B), and PRF1 (Perforin). In order to determine further which subpopulation increased in NK-like CD8+ T cells, we used CyTOF with a 26 marker phenotypic panel to evaluate over one million viable cells from the same individuals profiled in scRNA-seq analysis. As a result, we identified a population of NK-like CD8+ T cells that shared gene expression profiles with both NK cells and CD8+ T cells.

2985 Perinatal Lead (Pb) Exposure’s Effect on DNA Methylation in Human Umbilical Cord Blood

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Early life exposure to lead (Pb) can influence health through epigenetic modifications including DNA methylation. The aim of this study is to identify differentially methylated CpG positions associated with prenatal Pb exposure in human cord blood leukocytes. The Early Life Exposures in Mexico to Environmental Toxics (ELEMENT) project utilizes a series of longitudinal birth cohorts to examine the impact of Pb exposure during sensitive periods of development. In this study, we selected 97 ELEMENT participants with archived umbilical cord blood samples for methylation analysis via the Infinium MethylationEPIC BeadChip, which quantifies total methylation at ~850,000 CpG sites. Maternal blood lead levels (BLLs) at each trimester (T1: 6.56 ± 5.27 μg/dL; T2: 5.89 ± 4.83 μg/dL; T3: 6.20 ± 4.48 μg/dL), umbilical cord BLLs (4.82 ± 2.37 μg/dL) and maternal blood lead levels one-month post pregnancy were measured. Differentially methylated positions (DMPs) by Pb level were identified using linear regression, controlling for sex and estimated umbilical cord 

hypomethylates TE and this leads to increased expression of pIRNAs. We will discuss the functional implications of these alterations as a potential mechanism of multigenerational effects of environmental toxicants. Supported by NIH R01ES024915.
Tobacco smoke exposure is a risk factor for many human diseases and the global disease burden is attributed to it. In addition to DNA damage, smoking-induced epigenetic changes may contribute to etiology of complex smoking-associated diseases. Smoking alters the epigenome and transcriptome of human blood leukocytes. However, interpretation of bulk genomic approaches is limited because changes could indicate altered cellular mixtures or subpopulations. To characterize smoking-related gene expression changes in primary immune cells, we performed single cell RNA sequencing on human peripheral blood mononuclear cells (PBMCs) from smokers (n=4) and non-smokers (n=4). Transcripts of 45,965 cells (78,183 reads per cell) revealed an altered population of Natural Killer (NK)-like T lymphocytes in smokers. Compared to NK cells, the NK-like T cell cluster had elevated expression of CD8A, CD8B, CD3D, CD3E, CD3G, CD6, and CD2, indicative of CD8+ T lymphocytes. Recently rare in non-smokers (2.2%), the transcriptionally unique subset of CD8+ T cells comprised 8.7% of PBMCs in smokers. Among CD8+ T cell subtypes, the increase in NK-like CD8+ T cells (Mann-Whitney p = 0.03) corresponded with a decrease in Naïve CD8+ T cells (p = 0.03). We did not observe changes in the frequencies of two additional CD8+ T cell clusters or in the overall frequency of CD8+ T cells. Mass cytometry of a 26-antibody leukocyte panel confirmed no differences in the frequencies of total CD8+ T (CD3+CD8+ or CD8+ NK (CD3−CD8+)) or CD8+ NKT (CD3+CD8+) cells between smokers and non-smokers. This suggests smoking is associated with an increased number of CD8+ T cells that share characteristics with NK cells, but are not NKT cells. Consistent with an NK-like phenotype, altered effector CD8+ T cells had elevated expression of genes reported to be upregulated in NK cells reprogrammed toward NK cells. Compared to other effector CD8+ T cells, altered effector CD8+ T cells had reduced IL7R and increased FCGR3A (CD16), IFNG (interferon gamma), GZMB (granulysin B), and PRF1 (perforin) expression. In mice, granzyme B and perforin expressing CD8+ T cells contribute to the development of atherosclerotic plaques. Our data highlights a potential link between smoking-induced functional changes in human CD8+ T cells and atherosclerosis.
2990 Continuous Exposure to Morphine Sulfate Alters Genomic DNA Methylation in Mouse Embryonic Stem Cells

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Epigenetic changes alter heritable phenotypes without modifying DNA sequences and account for individual variability related to drug metabolism and response. This is particularly valid for drugs with addictive properties. Methylation and demethylation of genes also regulates organogenesis in mammalian cells. We have previously shown that morphine sulfate (MS) exposure for 24-hr induces hypomethylation of DNMT1, SA and 3B; i.e. methytransferases responsible for methylation of DNA under various conditions. In this study, we hypothesize that exposure to opioids for longer duration during fetal development affects pre-programmed genomic methylation and demethylation patterns associated with organogenesis, eventually leading to developmental abnormalities. To evaluate the epigenetic mechanism of action of morphine on the differentiation process of proliferating mouse embryonic stem (mES) cells, we monitored cytotoxicity, DNA methylation and cell viability after exposure to MS for 1-, 3-, 5- and 7-days. Cell viability using the MTT assay indicated cytotoxicity at a concentration of at least 1000 µM on day 1. A constant decrease in viability was observed for days 3, 5 and 7 at 1, 10, and 100 µM respectively. Further exposure of mES cells to the standard 10 µM resulted in hypomethylation for all time points evaluated. To confirm if these changes were heritable, cells were allowed to recover for 3 days in the absence of MS after each exposure period. Genomic methylation analysis after the recovery period revealed a reversal of MS-induced hypomethylation. Interestingly, hypermethylation of DNA was observed following 7-day exposure and 3-day recovery. Overall, the results disclose that MS induces heritable epigenetic changes with prolonged exposure at concentrations that do not significantly affect cell viability. Since DNA methylation is essential for maintaining embryonic progression and expansion of stem cells, our results suggest that MS negatively influences developmental progress by shifting DNA methylation patterns in differentiating cells.

2992 Fetal Epigenetic Reprogramming in Response to Early-Life Exposure to Phenols


Maternal exposure to environmental toxicants, including bisphenol-A (BPA), bisphenol-S (BPS), and bisphenol-F (BPF), during early pregnancy can impact an infant’s risk of later-life disease. Persistent changes in DNA methylation are a proposed mechanism through which the prenatal environment can impact lifetime health trajectory. In the Michigan Mother Infant Pairs (MIMP) Cohort, we assessed associations between maternal first-trimester urinary phenol concentrations and DNA methylation profiles in infant umbilical cord blood leukocytes (N=53). We used the Illumina Infinium MethylationEPIC Beadchip to quantitate DNA methylation across the epigenome at 822,609 CpG sites that passed quality checks. Urinary BPA, BPS, and BPF were adjusted for specific gravity (SG). BPS and BPF were dichotomized as detected versus non-detected, because 51% and 43% of samples had detectable concentrations, respectively. Models of single-CpG-site DNA methylation with BPA (continuous) or BPS and BPF (dichotomized) were adjusted for infant sex, maternal covariates, and estimated cord blood cell type proportions. In addition, differentially methylated regions (DMRs) were identified using DMRcate. Analysis with BPA revealed 47 CpG sites significant at a 5% false discovery rate, half of which exhibited less methylation with increasing BPA concentrations. Gene ontology revealed immune system processes, metal-sulfur cluster assembly, regulation of lyase, and succinate dehydrogenase activity as pathways enriched with differentially methylated genes. One DMR in the HOXA3 gene was associated with maternal BPA exposure. Analysis of maternal BPS and BPF exposure resulted in zero CpG sites below the 5% false discovery rate and zero DMRs associated with maternal BPF or BPS exposure. Thus, maternal exposure to BPA, but not BPF or BPS, was associated with differential methylation at the CpG and gene-region levels in infant cord blood. This analysis will be expanded to include DNA methylation profiling of matched placenta samples to evaluate tissue-dependent effects of phenols on the epigenome.

2993 Epigenetic Effects of Maternal Arsenic Exposure on Mice through Drinking Water


Both epidemiological investigations and animal studies have reported that long-term exposure to arsenic from drinking-water and food is associated with cancer, skin lesions, diabetes and developmental effects. However, a thorough understanding is lacking for how epigenetic patterns are perturbed by arsenic exposures. Thus in the present study, to characterize whether and how arsenic affects epigenetic patterns, 10 ppb arsenic were administrated to pregnant C57BL/6 mice until weaning through drinking-water. Body weight and liver function of the 5 months old offsprings were recorded. The results showed that maternal exposure to arsenic alter the body weight of the offspring, and significantly change their liver alamine aminotransferase activities. Transcriptome profiling by using mRNA sequencing revealed that 1222 genes were up-regulated and 614 genes were down-regulated in the offspring’s liver after maternal exposure to arsenic. Gene ontology analysis showed that these differentially expressed genes were enriched in immunological functions. Further examination on chromatin accessibility by using transposase-accessible chromatin sequencing (ATAC-seq) showed that open chromatin regions were significantly changed in the offspring upon maternal exposure to arsenic. Collectively, our investigation identified maternal arsenic exposure actually alters the offspring’s transcriptome via disrupting chromatin’s opening status.

2994 Investigation of the Effects of Zearalenone and Its Metabolites in the Genes Related to Energy Metabolism In Vitro: Relation with the Epigenetic Alterations

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Zearalenone (ZEA), produced by various Fusarium species, is a non-steroidal estrogenic mycotoxin. It contaminates cereals such as corn, wheat, oat and soybean. However, it has no negative effects on human health. In our study, we investigated the effects of ZEA and its metabolite α-ZOL on epigenetic modifications and metabolic pathways have been investigated in HepG2 cells in order to elucidate the possible relationship between metabolic dysfunctions. According to the MTT and NRU tests, the IC50 values were determined as 143.35 and 60.45 for ZEA and 111.42 and 35.72 µM for α-ZOL, respectively. At

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the concentrations of 1-50 µM of ZEA and α-ZOL exposures for 24 h were significantly increased cell proliferation by BrdU assay in the rates of 7.2-41.71% for ZEA, 11.78-44.33% for α-ZOL, respectively. 50 µM of ZEA and α-ZOL exposure caused significant alterations in the expression levels of nuclear receptor genes (AhR, LXRα, PPARα, PPARγ), alteration in PPAR expression and promoter DNA methylation significantly. Low Gestational Age Newborn (ELGAN) cohort. Our results highlight CpG methylation of critical genes including many that showed sex difference in placental CpG methylation in regards to prenatal acetaminophen exposure.

Reactive oxygen species (ROS) are direct regulators of many vital cellular and developmental processes. However, if left unchecked, excessive ROS can directly damage cellular macromolecules, so cells must maintain tight regulation of ROS. Following exposure to ROS, transcription factors (TFs) are activated and regulate the expression of a myriad of genes in an attempt to protect the cell from ROS-mediated damage and adapt to new environmental conditions. Such changes in gene expression patterns are highly dependent on the ability of TFs to bind DNA, and thus are directly impacted by the local chromatin state and DNA accessibility at target binding sites. For instance, some genes may be located in highly accessible chromatin regions that would allow for immediate ROS-responsive TF binding and gene activation, whereas others may require initial changes in DNA accessibility to allow for activation after long term ROS exposure. Our current models of ROS-induced changes in gene expression and DNA accessibility are fragmentary. Thus, we aimed to fill this gap in knowledge by exposing human MCF7 cells to two different ROS-inducing compounds (menadione [MEN] and tert-butyl hydroperoxide [tBOOH]), followed by transcription and DNA accessibility profiling at 0, 1, 8, or 24 hours. For measurement of gene expression we performed RNA-seq, and for DNA accessibility we utilized the Assay for Transposase-Accessible Chromatin followed by high throughput sequencing (ATAC-seq). We found that MEN exposure resulted in significant changes in expression (FDR-corrected $p < 0.05$) of 38 genes after 1 hour of exposure, an additional 1676 genes at 8 hours, and another 937 genes at 24 hours. Using fine mapping and computational methods to determine transcription factor binding, we identified 38 genes with increased TF binding and DNA accessibility after MEN exposure and opening and closing of chromatin due to ROS exposure. In contrast to the dramatic and dynamic changes in gene expression, we detected a comparatively small number of significant changes in DNA accessibility (<50) across the genome. Similar trends in both gene expression and DNA accessibility were observed with tBOOH treatment. These results suggest that large scale changes in chromatin remodeling is not necessary to mount ROS-induced oxidative stress. Instead, we found that DNA accessibility changes are necessary for transcriptional response to oxidative stress. In summary, our data support a model in which transcription factors are recruited to perform transcriptional reprogramming by oxidative stress. Instead, our data support a model in which transcription factors are recruited to perform transcriptional response to oxidative stress.
the liver. This, although it might be depend on the characteristics of drugs, suggests that eye-drop administration does not always induce more severe genetic changes in corneas than in systemic target organs.

2999 Potential Candidate Genes Driving Cancer Stemness and Their Regulation by Novel Triterpenoids in Triple Negative Breast Cancer

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Triple negative breast cancer (TNBC) is a subtype of breast cancer that is prone to metastasis and high rates of recurrence. Cancer stem cells, responsible for metastasis and resistance to chemotherapy, have been shown to be highly enriched in TNBC. Therefore, treatment of cancer cells with novel pharmacologic agents targeting cancer stemness have the potential to mitigate TNBC’s aggressive phenotype. CDDO-Imidazole (CDDO-I, 1-(2-cyano-3,12-dioxoooleana-1,9-diene-28-yl) imidazole) is a synthetic triterpenoid previously shown to reduce cancer stemness in TNBC cells. The purpose of this study was to identify potential candidate genes important in cancer stemness, and whether CDDO-I treatment would regulate the expression of those genes to inhibit TNBC. The human TNBC cell line SUM159 was used to prepare mammospheres, which are designed to enrich for cancer stem cells. To identify potential candidate genes driving stemness, RNA sequencing analysis was performed on SUM159 cells in monolayer culture, mammospheres, and mammospheres treated with CDDO-I. The samples were subjected to GSEA analysis along the gut-brain axis. The AhR and its ligands also inhibit colon cancer stem cells include TNFa, TGF-β, HIFa, and EGF, all of which are integral to proliferation. Additional novel genes found to be upregulated with enriched cancer stem cells include LOXL2, which promotes cancer cell stemness and stemness in breast cancer, and SLC2A3, which has a role in self-proliferation in embryonic stem cells, and these genes are downregulated when treated with CDDO-I. One of the major pathways downregulated when cells were treated with CDDO-I was the TNFa pathway via HMox1, which was further validated by quantitative PCR analysis. These results suggest that several novel genes may have a significant role in regulating cancer stemness in TNBC, as well as the potential of CDDO-I to reduce the cancer stem cell phenotype of TNBC, and therefore potentially provide better therapeutic outcomes.

3000 The Aryl Hydrocarbon Receptor Is a Tumor Suppressor-Like Gene in Glioblastoma

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The aryl hydrocarbon receptor (AhR) plays an important role in maintaining cellular homeostasis and in pathophysiology, and the interplay between the gut microbiome and microbial-derived AhR ligands protect against inflammation along the gut-brain axis. The AhR and its ligands also inhibit colon carcinogenesis, whereas it has been reported that the AhR and its ligand kynurenine enhance glioblastoma (GBM). In this study, we have re-examined the role of kynurenine and the AhR in GBM using established and patient-derived GBM cells at the functional, molecular and genomic levels. In all the cell lines, kynurenine did not modulate AhR-mediated gene expression and did not affect invasion of GBM cells. Moreover, based on results of transient and stable (by CRISPR/Cas9) AhR knockdown in GBM cells, we observed that loss of AhR enhanced GBM cell and tumor growth, enhanced GBM cell invasion, and increased expression of pro-invasion/pro-migration genes as determined by IPA analysis of RNAseq data. Thus, the AhR is a tumor suppressor gene in GBM and therefore a potential drug target for patients that express this receptor.

3001 Remodeling of Plasma Membrane Proteolipid Composition by Environmental Chemicals and Membrane-Targeted Dietary Bioactives

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Abrupt activation of signaling through the epidermal growth factor receptor (EGFR)/Ras pathway is implicated in many cancers. EGFR and Ras signaling originates from transient nanoscale compartmentalized regions of the plasma membrane composed of specific proteins and lipids. The highly specific lipid composition of these nanodomains, termed nanoclusters, facilitates receptor recruitment and therefore influences signal transduction. This suggests that nanocluster proteolipid composition and formation could represent a site of action for environmental chemicals and a novel target for future chemoprevention interventions. Exposure to the environmental chemical bisphenol A (BPA) can activate EGFR/Ras signaling, while consumption of fish containing long-chain n-3 polyunsaturated fatty acids (n-3 PUFA) such as eicosapentaenoic acid (EPA, 20:5n-3, 8,11,14,17) and docosahexaenoic acid (DHA, 22:6n-3,7,10,13,16,19) can reduce EGFR/Ras signaling, yet the mechanism underlying these effects is unknown. We hypothesize that environmental chemicals that activate EGFR/Ras signaling, modulate the proteolipid composition of EGFR and Ras nanoclusters and that n-3 PUFA can block this effect. Here we demonstrate that dietary n-3 PUFA reduce the lateral segregation of cholesterol-dependent and -independent nanoclusters, suppressing phosphatidic acid-dependent oncogenic KRas effector interactions, via their physical incorporation into plasma membrane phospholipid. This results in the inhibition of oncogenic Ras-driven colonic hyperproliferation in both Drosophila and murine models. Future studies will characterize the effect of BPA on EGFR nanocluster formation. These findings demonstrate the unique properties of dietary n-3 PUFA in shaping plasma membrane nanoscale proteolipid complexes and support the emerging role of plasma membrane-targeted therapies.

3002 Vitamin D Receptor Expression Modulates the Angiogenic Properties of Endothelial Cells through Suppression of Oxidative Stress and Inflammation

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Vitamin D is an important modulator of angiogenesis and tumor growth. We previously showed the active form of vitamin D, 1,25(OH)2D3, is a potent inhibitor of retinal neovascularization. How vitamin D inhibits retinal neovascularization and whether these activities are mediated through vitamin D receptor (Vdr) remains of significant interest. Vitamin D receptor (VDR) is a nuclear receptor that mediates the majority of molecular events associated with vitamin D action. However, the role of VDR expression in retinal angiogenesis, and more specifically in retinal endothelial cell (EC) function remain unknown. Here we demonstrate that VDR expression in retinal EC function remain unknown. Here using retinal EC prepared from wild type (Vdr+/+) and Vdr-deficient (Vdr-/-) mice, we investigated the impact of VDR expression in retinal EC function. Lack of VDR had a significant impact on endothelial cell-cell and cell-matrix interactions. Vdr-/- retinal EC showed altered levels of vascular endothelial cadherin and occludin-1, affecting the formation of adherens and gap junctions. However, the ability of Vdr-/- retinal EC to undergo capillary morphogenesis on Matrigel was minimally affected. Vdr-/- retinal EC proliferated at a slower rate than their wild-type counterparts. Vdr-/- retinal EC exhibited increased expression levels of inflammatory markers including osteopontin and monocyte chemoattractant protein-1. Vdr-/- retinal EC also showed increased reactive oxygen species generation following hydrogen peroxide challenge. These changes were attributed, in part, to sustained activation of mitogen-activated protein kinase (MAPK) pathways, enhanced nuclear localization of NF-κB, and down-regulation of endothelial nitric oxide synthase expression in Vdr-/- retinal EC. Taken together, our results indicate that VDR expression has a significant impact on oxidative stress and inflammation, which contributes to regulation of angiogenic properties of EC.

3003 Decoding Aryl Hydrocarbon Receptor-Mediated Differential Gene Expression

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The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that is primarily studied as a regulator of xenobiotic metabolism, most notably through the activation of cytochrome P450 1A1 expression in response
Triclosan (TCS) is a popular wide-spectrum antimicrobial agent. While TCS was recently banned from several consumer products such as hand soaps, it remains in a top-selling toothpaste and in other household items. TCS inhibits the functions of both mast cells and also of mitochondria. Mast cells are found in most human tissues and play important roles in a wide array of biological processes and diseases. Through investigating the mechanisms by which TCS disrupts mitochondrial and mast cell signaling, the Gosse lab found that TCS depolarizes the mitochondrial membrane and disrupts cellular Ca\(^{2+}\) dynamics. However, triclosan’s effects on mast cells and mitochondria disappear when its ionizable hydroxyl group is not present. These findings suggest TCS to be a proton ionohore capable of not only uncoupling mitochondria, but also possibly capable of disrupting the plasma membrane electrochemical potential (PMP). In this study, we have utilized a fluorescent, genetically-encoded voltage indicator called ArcLight A242 to probe TCS effects on mast cell PMP. Utilizing this method for the first time in mast cells, we observed plasma membrane depolarization in the presence of gramicidin, using confocal microscopy coupled with image analysis. Using these newly-developed methods, preliminary results show that TCS indeed inhibits PMP of mast cells. TCS disruption of PMP could provide a mechanistic explanation of triclosan’s disruption of Ca\(^{2+}\) influx, mast cell function, and a host of other cellular processes dependent on PMP.
Currently mechanistic understanding of drug-induced liver injury (DILI) is still lacking and therefore hard to predict during drug development. Some DILI compounds are known to induce endoplasmic reticulum (ER) perturbations. During chronic ER stress, activation of the adaptive unfolded protein response (UPR) is insufficient and will activate apoptotic pathways mediated by CHOP leading to hepatotoxicity. This switch from adaptive to adverse signaling is poorly understood. To improve mechanistic understanding of the UPR, we aimed to identify novel key regulators of CHOP. First, the suitability of the HepG2 CHOP-GFP reporter for evaluating CHOP induction was determined using a concentration range of various ER stress inducers. Secondly, the most optimal ER stress condition was defined where a concentration of 6 μM of tunicamycin was found most optimal to study CHOP induction perturbations by siRNA-mediated knockdown. Using these conditions, we applied an imaging-based RNA screen of the druggable genome targeting 3457 genes (including kinases, ubiquitinases, transcription factors and epigenetic factors) in HepG2 CHOP-GFP cells to identify novel regulators of the tunicamycin-induced ER stress response. CHOP-GFP expression was evaluated after 16 hours of exposure to 6 μM of tunicamycin which was altered by 201 genes upon knockdown from which 74 could be confirmed. These potential regulators were further evaluated with other ER stress inducers and their role in induction of other UPR-related genes such as ATF4, XBP1 and Bip. To evaluate the relevance of 10 selected novel regulators for the human liver during ER stress, we evaluated the gene expression using TempO-seq transcriptomics technology in both HepG2 and PHHs after knockdown and subsequent exposure for 16 hours of tunicamycin. Three potential regulators were confirmed in PHH which showed upon knockdown perturbation of UPR activation after tunicamycin. Next, their role in DILI compound-induced ER stress was determined, where potential regulators could affect significantly the UPR activation and cell death induction during DILI compound exposure. Overall, our RNAi screen allowed the identification of novel regulators of the drug-induced ER stress response and will further shape our understanding and prediction of DILI liabilities. Supported by EU-ToxRisk project (grant agreement No 681002) and IMI-MIP-DILU project (grant agreement 115330).

**EGFR Activates the NRF2-KEAP1 Signaling Pathway in a Novel Mechanism Involving Cellular Localization Changes of FAM129B**

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Resistance to chemotherapies and radiotherapies is an increasing issue among cancer patients. One mechanism by which these tumors can confer resistance to therapies is via an upregulation of redox regulating proteins as a result of induction of the nuclear factor (erythroid-derived 2)-like 2 (NRF2) signaling pathway. Canonical activation of NRF2 requires modification of key cysteine residues in its negative regulator, Kelch-like ECH-associated protein 1 (KEAP1), that results in NRF2 heterodimerizing with MAFG, binding to antioxidant response elements (AREs), and recruiting transcriptional machinery. It has been well demonstrated that the NRF2-KEAP1 signaling pathway is activated in response to reactive oxygen species (ROS); however, here we show a novel mechanism by which NRF2 is induced independently of ROS and cysteine oxidation. In these investigations we elucidate the mechanism by which family with sequence similarity 129, member B (FAM129B) induces NRF2 in cancer cells. We demonstrate that activation of epidermal growth factor receptor (EGFR) directly phosphorylates FAM129B, thus causing a shift in localization of the protein from the plasma membrane to the cytosol. Upon movement to the cytosol, FAM129B competitively binds to KEAP1 via its ETGE motif, thus resulting in translocation of NRF2 to the nucleus and activation of transcription. In this study, we show that mechanistically lead toxicity further upregulates NRF2 by activating EGFR, thus leading to the phosphorylation of FAM129B. Overall, we characterized a novel mechanism by which EGFR activation causes phosphorylation and thus changes in cellular localization of FAM129B, thus allowing binding to KEAP1 and induction of NRF2, which could be relevant to conferred resistance to cancer therapies.

**Digital Spatial Molecular Profiling of H&E or Antibody Stained FFPE: Measurement of Complex Gene Profiles within the Context of the Tissue Microenvironment**

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Measurement of complex gene expression molecular signatures from fixed tissue has been problematic, especially given the desire to correlate those measurements to existing histology techniques within the context of the tissue microenvironment. We report the use of the Digital Spatial Profiling (DSPM) assay, an in situ adaptation of the TempO-seq™ targeted gene expression analysis on FFPE or IHC stained slides from histologically defined regions of interest down to a spatial resolution of 20μm. FFPE sections were processed on an automated slide stainer (Bond RX/m) where they are infused with TempO-seq probes and stained. The antibody- or H&E-stained slides were then digitally imaged and TempO-seq probes automatically eluted from selected regions of interest using the CellSensus platform, followed by qPCR amplification, pooling of the samples, sequencing, and automated analysis using the TempO-seq™ software package. The H&E staining not only enabled identification of the areas of tissue histology to be profiled, but provided images that validated that the profiling data was tissue specific and not cross-contaminated. Using a pan-cancer assay of 5,207 genes that included the S1500 v2 surrogate assay, we profiled discrete focal areas of stroma, normal epithelium, cancer epithelium, and pre-cancerous high-grade PIN lesions from H&E stained archived prostate cancer patient resections. The data demonstrate not only that the different tissues within the FFPE section can be profiled without contamination by neighboring cell types, but demonstrate an unexpected level of biomarker specificity as a result of the spatial resolution of the assay. Many genes expressed by normal epithelium were not expressed in cancer or stroma, and many genes expressed in stroma were not expressed in cancer or normal. We hypothesize that spatial resolution provides biomarker specificity that is not seen in heterogeneous tissue assays, and speculate that there are factors in tissue which cause specificity, whether it is adjacent cells, the structural effects of tissue, or chemical factors segregated within the tissue, which are not present in cultured or isolated cells.

**Mechanism of Rapid Induction of Interleukin 22 in Dendritic Cells and Its Modulation by AhR Ligands**

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The expression of interleukin 22 (IL-22) has been primarily associated with lymphoid cells including activated T cells and innate lymphoid cells (ILCs). Here we show that bone marrow-derived dendritic cells (BMDC) express and produce IL-22 after activation of the aryl hydrocarbon receptor (AhR) when cells are activated by agonists of the toll like receptor (TLR) family. The additional activation of AhR is necessary for a significant induction of IL-22 in TLR activated BMDC, while polyinosine-polyribonucleotide (P(LPS)-treated B6 mice. The results show that induction of IL-22 expression via AhR is independent from RORγt or IL-23. We identified four consensus dioxin responsive elements (DRE), one Reib/AhR (ReLB/AhRE) binding element and a consensus NF-κb site in the first 3500 bp upstream of the transcriptional start site of the mouse il22 gene. Deletion and mutation constructs of the IL-22 promoter revealed that the NF-κb consensus element and two DRE consensus elements upstream of the start site are necessary to mediate the synergistic effects of AhR and TLR ligands in BMDC. Inhibitor studies and BMDC derived from knockout mice confirmed that the induction of IL-22 by AhR and TLR ligands depend on the expression of AhR as well as Reib, but was not affected by the inhibition of RORγt or IL-23. In summary, these results show that simultaneous activation of AhR and TLR signaling rapidly induce IL-22 in BMDC and the synergistic induction of IL-22 in BMDC may differ from lymphoid T cells which require RORγt.

**Assessing the Role of Arsenite in Disrupting the EGFR Signaling Axis**

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The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase localized on the cell surface. Overexpression of EGFR has been used as biomarkers for many different types of cancers, including lung cancer. Though there is a strong association between arsenic and lung cancer development,
the clear mechanism is unknown. We hypothesize that chronic arsenite exposure disrupts the EGFR endocytic trafficking, leading to increased receptor expression. The goal of this project is to study impact of chronic exposure to "a physiologically relevant" level of arsenite on the EGFR expression, distribution and trafficking. A non-malignant human bronchial epithelial cell line, Beas-2B cells were exposed to 100nM arsenite for 24 weeks. The chronic arsenite-treated cells showed decreased EGFR protein expression levels and activity, increased transcription levels of TGFα, and altered the distribution of the EGFR. In conclusion, the impact of chronic arsenite exposure on the EGFR signaling axis can explain arsenite-induced overexpression of the EGFR that is commonly characterized in lung cancer.

3013 TCDD-Inducible Poly-ADP-Ribose Polymerase (TIPARP) Catalytic Activity Protects against Dioxin-Induced Hepatotoxicity and Lethality

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We have previously reported that whole body or hepatoocyte specific deletion of TCDD-inducible poly-ADP-ribose polymerase (TIPARP), an AhR target gene and mono-ADP-ribosyltransferase, increases the sensitivity of mice to dioxin-induced toxicities. Our in vitro data show that TIPARP functions as part of a negative feedback loop to repress AhR signaling in a mechanism that requires TIPARP's catalytic activity. We hypothesized that the loss of TIPARP catalytic activity would be sufficient to increase sensitivity to TCDD-induced toxicity in vivo. To test the hypothesis, we used CRISPR/Cas9 gene targeting to generate a histidine 532 to alanine A mutation in TIPARP, creating a catalytically deficient mouse (Tiparp<sup>H532A</sup>). Hepatocytes isolated from Tiparp<sup>H532A</sup> mice had increased AhR target gene expression compared with wild-type. Tiparp<sup>H532A</sup> mice given a single injection of 100 or 10 μg/kg dioxin did not survive beyond day 3 and 10, respectively. All wild-type mice survived the 30-day treatment. At day 6, dioxin (10 μg/kg) treated Tiparp<sup>H532A</sup> mice exhibited increased expression of AhR target genes, higher levels of several inflammatory cytokines and chemokines, increased body weight loss, reduced white adipose tissue, decreased hepatic and gut toxicity as indicated by increased alanine aminotransferase activity compared with wild-type mice. RNA-Sequencing identified 647 genes significantly different after dioxin treatment in wild-type mice compared with 4551 genes significantly different in similarly treated Tiparp<sup>H532A</sup> mice. These data illustrate that loss of TIPARP's catalytic activity is sufficient to increase AhR signaling and increase sensitivity to dioxin toxicity. Our findings support TIPARP and mono-ADP-ribosylation as important negative regulators of AhR signalling pathway.

3014 Characterization of the Aryl Hydrocarbon Receptor-Kruppel-Like Factor 6 Interaction

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The aryl hydrocarbon receptor (AhR) is a transcription factor responsible to regulate toxic responses of chemical pollutants, including polycyclic aromatic hydrocarbons (PAHs) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). In the classical pathway, upon ligand binding, AhR translocate to the nucleus, dimersize with the Aryl Hydrocarbon Receptor Nuclear Translocator (ARNT) after releasing the chaperons Hsp90, Aip and Ara9. AhR/ARNT binds to DNA regions known as Xenobiotic Response Elements (XREs) facilitating the transcrip- tion of target genes. Our published results demonstrated that the AhR is also involved in a novel protein-DNA complex with the Kruppel Like Factor 6 (KLF6), a zinc-finger transcription factor related to SP1 that is involved in transcription of target genes. Our published results demonstrated that the AhR is also involved in a novel protein-DNA complex with the Kruppel Like Factor 6 (KLF6), a zinc-finger transcription factor related to SP1 that is involved in transcription of target genes. AhR and KLF6 complex is independent of XRE, targeting a non consensus XRE (AhR-KLF6 complex is independent of XRE, targeting a non consensus XRE). Further, a discrete sub-G1 population (18%) was unique to AhR<sup>-/-</sup> mice and returned CYP1A1 activity to control levels (p<0.05). 23C was also able to increase CYP1A1 to control levels, while 23A decreased the effects elicited by FICZ by 35% in Hepa1c1c7 cells (p<0.05). Importantly, all three compounds (5-20 μM) did not alter cell number following examination in a sulfurdihemate B assay. Thus, the change in CYP1A1 was not related to changes in cell number following treatment with the compounds. The results indicate that these marine-derived indoles may be able to reduce the toxicity caused by halogenated aromatic hydrocarbons. However, further direct aryl hydrocar- bon receptor binding studies are required to confirm these findings.

3015 Halogenated Indoles from Seaweed Act as Aryl Hydrocarbon Receptor Antagonists in Hepa1c1c7 Cells

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A range of polyhalogenated indoles have been isolated from Rhodophyllus membranacea collected in the Wellington harbor in New Zealand. The three most abundant indoles isolated were 4,7-dibrom-2,3-dichloroindole (23A), 7-bromo-2,3-dichloro-6-iodoindole (23B) and 6,7-dibrom-2,3-dichloro-2,3-dichloroindole (23C). These three compounds were then evaluated for their ability to induce CYP1A1 in Hepa1c1c7 cells and HepG2 cells. From ERD assays showed that only 23A (25-50 μM) induced CYP1A1 catalytic activity (3.5-fold) following 6-24 h exposure in Hepa1c1c7 cells and this effect was comparable to the EROD activity induced by 100 nM 5,11-dihydro-indolo[3,2-b] carbazole-6-carboxaldehyde (FICZ). In contrast, 23A, 23B and 23C (5-20 μM) were not able to significantly reduce CYP1A1 activity induced by 100 nM FICZ. Specifically, 23B (5 μM) + FICZ (100 nM) completely blocked FICZ induction and returned CYP1A1 activity to control levels (p<0.05). 23C was also able to decrease CYP1A1 to control levels, while 23A decreased the effects elicited by FICZ by 35% in Hepa1c1c7 cells (p<0.05). Importantly, all three compounds (5-20 μM) did not alter cell number following examination in a sulfurdihemate B assay. Thus, the change in CYP1A1 was not related to changes in cell number following treatment with the compounds. The results indicate that these marine-derived indoles may be able to reduce the toxicity caused by halogenated aromatic hydrocarbons. However, further direct aryl hydrocarbon receptor binding studies are required to confirm these findings.

3016 Distinct Roles for Xenopus laevis AhR Paralogs in Cell Cycle Regulation


The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that mediates toxicity of dioxin-like compounds. AhR also plays roles in de- velopment and physiology in the absence of xenobiotics. These include pro motion of cell cycle progression through the G1/S checkpoint. Unlike humans and rodents, the frog Xenopus laevis has two AhR paralogs, AhRα and AhRβ, the result of a genome duplication ~34 mya. We recently generated mutant derivatives of the XLK-WG cell line, each lacking a functional version of either AhRα or AhRβ, and showed that the two receptors mediate distinct transcriptional responses to specific agonists. AhRα<sup>−/−</sup> mutants also require longer time to reach confluency. This study tests the hypothesis that each AhR plays a distinctive role in the regulation of cell cycle progression. To quantify differences in the proliferation of wild type and AhR knockout cell lines, we measured growth using the water-soluble formazan (WST-8) method. After 96 hours, XLK-WG and AhR<sup>−/−</sup> cells displayed nearly identical growth, whereas AhR<sup>α<sup>−/−</sup></sup> cultures contained approximately 30% fewer cells. We determined cell cycle distribution of each line by propidium iodide staining and flow cytometry. AhR<sup>β<sup>−/−</sup></sup> and wild type lines exhibited similar distributions of cells in the various phases (~ 5% in G0/G1, 50% in S, and 15% in G2. In contrast, only 16% of AhRα<sup>−/−</sup> cells were in S-phase, and only 7% in G2. Further, a discrete sub-G1 population (18%) was unique to AhRα<sup>−/−</sup> cells. These results are consistent with a distinctive role for AhRα in cell cycle regu- lation. However, AhRα<sup>−/−</sup> is approximately 3-fold more abundant than AhRβ<sup>−/−</sup> and its more severe knockout phenotype may stem from a greater overall reduction in cellular AhR content. Ongoing investigations seek to determine whether cell cycle regulation effects are caused by different expression lev- els of the AhR paralogs or truly distinct functions. Most vertebrates have multiple AhRs. This study contributes to understanding the differences between each receptor and how their expression affects interpretation of data from non-mammalian toxicological models.

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3018 Hepatic Loss of Aryl Hydrocarbon Receptor (AhR), but Not AhR Nuclear Translocator, Mitigates High-Fat Dietary Challenge

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The ligand-activated aryl hydrocarbon receptor (AhR) is a well characterized membrane receptor that responds to xenobiotic compounds, transforming proteins, and certain endogenous compounds. The AhR normally resides within a cytosolic protein complex that translocates to the nucleus upon AhR-agonist interaction. Following nuclear localization, the AhR dissociates from the complex to form a DNA-binding heterodimer with aryl hydrocarbon receptor nuclear translocator (ARNT). The AhR-ARNT dimer then binds to canonical and non-canonical xenobiotic response elements (XRE) to activate gene transcription. Our previous work has revealed that the AhR can also activate gene expression independent of ARNT, through interactions with Kruppel-type factor 6 (KLF6) at non-canonical XRE sites comprised of 5′-GGGA-3′ repeats. Here, we examined the effects of KLF6-specific AhR or ARNT loss in high-fat dietary (HFD) chow-induced, inducible conditional knockout mouse models that allows for the timed deletion of AhR or ARNT in adult mice. The data demonstrate that AhR loss significantly reduces HFD-driven weight gain by 59% compared to control mice, and significantly lowers fasting glucose by 28%. AhR deficiency additionally decreases hepatic lipid deposition and adiposity within perigonadal white adipose tissue (gWAT). Hepatocyte-targeted deletion of ARNT reduces hepatic lipid deposition, but does not alter HFD-driven weight gain or fasting glucose, and significantly increases qWAT adiposity 26%. Next-Generation Sequencing analyses reveal that only 3% of gene transcripts are down- or up-regulated in both HFD-fed AhR KO and ARNT KO mice. The data suggest that the protective effects of AhR loss against HFD challenge may be partly mediated via a loss of ARNT-independent signaling and highlight the need to further study this type of AhR activity. Current work is under way to identify specific gene pathways through which the AhR influences energy metabolism independent of ARNT. This work is supported by grants R01ES026874 and T32ES007254.

3019 Characterizing Compounds from the Tox21 10K Compound Library as Activators of the Human Constitutive Androstane Receptor

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The constitutive androstane receptor (CAR; NR13) is a nuclear receptor which plays a significant role in all phases of drug metabolism and disposition. Recently it has also been implicated in modulating energy metabolism and different aspects of multiple cancer pathways. Therefore, identifying potential inducers of hCAR activation could allow for the prediction of potential therapeutic usage or drug-drug interactions. We screened the Tox21 10,000 compound collection to characterize potential hCAR activators. The screen revealed four compounds, rimacazole, dimepanid, phenothrin, and neticazole, as potential novel hCAR activators that also mediate CAR translocation from the cytoplasm to the nucleus of hepatocytes, which is the first step of CAR activation. One of these four compounds, rimacazole, demonstrated selectivity toward hCAR suggesting the need for further studies to establish the complete profile of this drug in the body. Categorized as hCAR activators, these compounds could be developed as potential therapeutic drugs, but are also at risk for drug-drug interactions.

3020 Aryl Hydrocarbon Receptor-Mediated Changes in Histone Mobility

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The Aryl Hydrocarbon Receptor (AhR) is a ligand mediated transcription factor which regulates response to xenobiotics, including 2,3,7,8-tetrachlorodibeno- p-dioxin (TCDD). AhR signaling is mediated via xenobiotic response elements (XREs) found within the promoters of AhR target genes such as CYP1A1 and CYP1B1. Our laboratory has previously reported a distinct non-consensus xenobiotic response element (non-XRE) that also confers AhR-mediated target gene expression. AhR binding to NC-XRE sites in response to TCDD involves recruitment of both Kruppel-like Factor 6 (KLF6) and Carbamoyl Phosphate Synthase 1 (CPS1). CPS1 recruitment to the chromatin results in homocitrullination (H1K34hCIT), a novel epigenetic histone mark. The H1K34hCIT modification is agonist specific, occurring only in response to exogenous agonists such as TCDD, but not endogenous agonists such as cinnabarinic acid. We hypothesize the H1K34hCIT modification increases histone mobility in NC-XRE containing regulatory regions, to promote chromatin accessibility of the transcriptional initiation complex. Western blot analyses of nuclear extracts from TCDD-treated mouse liver demonstrate increased levels of H1K34hCIT. In vivo chromatin immunoprecipitation confirms the presence of the AhR, CPS1 and the H1K34hCIT modification at NC-XRE regulatory elements in known, non-canonical AhR target genes. Changes in nucleosome mobility and position in response to TCDD were evaluated genome-wide using Micrococcal Nuclease (Mnase)-seq. Here, we report that TCDD induces a significant increase in the amount of H1K34hCIT-risking Mnase fragments at the promoters of AhR target genes in response to TCDD. This work highlights the evolving intricacies of AhR biology and cross-talk with the epigenome. This work is supported by NIH grant R01ES026874.

3021 Triphenyl Phosphonate, an Environmental Contaminant, Is a Selective PPARy Ligand That May Not Be So Brite

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Metabolic homeostasis is regulated, in part, by a family of proteins called nuclear receptors through which lipophilic hormones and molecules regulate gene transcription. One such nuclear receptor is the peroxisome proliferator activated receptor y (PPARy). Its activation is essential for white, brite (brown in-white), beige and brown adipogenesis, mature adipocyte maintenance, and insulin sensitivity. Accumulation of white adipocytes significantly increases the risk of metabolic syndrome. On the other hand, brown and brite adipocytes potentially counteract metabolic disease-related symptoms. The white adipogenic, brite/brown adipogenic, and insulin sensitizing activities of PPARy are regulated separately through differential post-translational modifications and/or co-repressor recruitment, with ligands having distinct abilities to activate each of PPARy’s functions. In differentiated 3T3 L1 cells, triphenyl phosphonate (TPHP; a flax antibiotic, 20 μM) induces an adipocyte phenotype that is distinct from rosiglitazone (Rosi, a therapeutic PPARy ligand, 20 μM) and roscovitine (Rosco, a CDK5 inhibitor, 10 μM). While only TPHP and Rosi enhanced hormone-stimulated adipogenesis, as evidenced by the significant increase in lipid accumulation and gene expression of mature adipocyte markers (Ppard, Ppara, Cidea, Elov5). In addition, only Rosi and Rosco protected PPARy from phosphorylation at Ser-273. In mutated 3T3 L1 cells in which PPARy cannot be phosphorylated, TPHP is able to induce mRNA expression of Ucp1 and reduce Fabp4 expression. Moreover, short term (2 weeks) in vivo exposures to Rosi, TPHP, or Rosco in 8-week female C57BL/6J mice caused a significant increase in brite adipocyte gene expression (Elov5; Ucp1) in mature adipocytes from Rosi- and Rosco-treated mice and an increase in white adipocyte gene expression (Plin1, Retn) in mature adipocytes from TPHP-treated mice. These data support our hypothesis that environmental PPARy ligand, TPHP, skew...
adipocyte differentiation toward white adipogenesis at the expense of brite adipogenesis, potentially through modifying post-translational modifications of and/or co-regulator recruitment by PPARy.

**3022 The AhR Regulates Glycolysis to Promote SIRT1-Dependent Keratinocyte Differentiation**


Activation of the aryl hydrocarbon receptor (AhR) by the ligand, 2,3,7,8-tetrachlorodibenzodioxin (TCDD) increased AhR binding in the promoters of the glucose transporter, SLCA2A1, and the glycolytic enzyme, enolase 1 (ENO1). Chromatin binding of the AhR corresponded to decreases of SLCA2A1 and ENO1 mRNA, protein and activities. Measurements of the ENO1 promoter in reporter assays demonstrated that activation of the AhR decreased the transcription of ENO1. Glycolysis was lowered by activation of the AhR as measured by decreases in glucose uptake and the production of pyruvate and lactate. Down-regulation of glucose metabolism, either by activation of the AhR or inhibition of glycolysis, increased the level of SIRT1 protein in NIH3T3 cells. This increase of SIRT1 was inhibited by the addition of exogenous pyruvate. Moreover, keratinocyte differentiation in response to decreased glucose metabolism was dependent on SIRT1. These results indicate that keratinocyte differentiation is regulated by cellular metabolism and that ligand-mediated activation of the AhR decreases the expression of SLC2A1 and ENO1, thereby decreasing glycolytic flux and controlling the fate of keratinocytes.

**3023 Phenobarbital is an Agonist of Human but Not Mouse PXR**

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Phenobarbital (PB), a broadly used anti-seizure drug, was the first to be characterized as an inducer of hepatic cytochrome P450 (CYP) by the activation of the constitutive androstane receptor (CAR, NR1I3). While PB is recognized as a prototypical CAR activator through a well-documented indirect activation mechanism, conflicting results have been reported regarding whether PB can activate the orphan X receptor (PXRx, NR1I2), a sister receptor of CAR, across different species, and the underlying mechanisms are largely unknown. Here, we show that in the human CAR (hCAR)-knockout HepG2 cell line, both PB and rifampicin (a selective hPXRx activator)-mediated induction of CYP3A4 and CYP2B6 was intact while induction by CITCO (a selective hCAR activator) was significantly attenuated. In human primary hepatocytes and HepG2 cells, knockdown of PXRx by recombinant lentivirus or co-treatment with SPA70, a potent antagonist of hPXRx, inhibited CYP3A4 induction by PB and rifampicin. PB showed concentration-dependent activation of hPXRx but not mouse PXRx (mpXR) in HepG2 transactivation assays, while such activation was fully abolished by introducing a non-functional hPXRx mutant. Mechanistically, our surface plasmon resonance binding affinity assay showed that PB directly binds to the ligand binding domain (LBD) of hPXRx (KD (M) = 1.42E-05). PB also activates hPXRx but not mPXRx and provides an alternative pathway for PB-mediated CYP induction.

**3024 AhR2, but Not AhR1α or AhR1β, Is Required for Craniofacial and Fin Development and TCDD-Dependent Cardiotoxicity in Zebrafish**

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The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that binds environmental toxins and regulates gene expression. AhR also regulates developmental processes, like craniofacial development and hematopoiesis, in the presence of environmental exposure. Zebrafish have three paralogues of AhR: AhR1α, AhR1β and AhR2. Adult zebrafish with mutations in AhR2 exhibited craniofacial and fin defects. However, the degree to which AhR1α and AhR1β influence AhR2 signaling and contribute to fin and craniofacial development are not known. We generated zebrafish with mutations in each AhR gene and compared morphology of adult AhR2 mutant and AhR1α/AhR1β single and double mutant zebrafish. We found that AhR1α/AhR1β single and double mutants were morphologically normal while AhR2 mutant zebrafish demonstrated fin and craniofacial malformations. At 5 days post fertilization, both AhR1α/AhR1β and AhR2 mutant larvae were normal, suggesting that adult phenotypes are due to defects in maturation or maintenance. AhR was shown to interact with estrogen receptor alpha, yet it is not known whether these interactions are constitutive or dependent on AhR1 genes. To determine whether estrogen receptors are constitutive co-factors for AhR signaling, we used genetic and pharmacologic techniques to analyze TCDD-dependent toxicity in estrogen receptor and AhR mutant embryos. We found that embryos with mutations in AhR1α/AhR1β or estrogen receptor genes are susceptible to TCDD toxicity while AhR2 mutant embryos are TCDD-resistant. Moreover, pharmacologic blockade of nuclear estrogen receptors failed to prevent TCDD toxicity. These findings suggest that AhR1 genes do not have overlapping functions with AhR2 in fin and craniofacial development or TCDD-dependent toxicity, and that estrogen receptors are not constitutive partners of AhR2.

**3025 AhR Signaling Regulation by ARNT Isoform Modification and Co-regulator Recruitment**

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The aryl hydrocarbon receptor (AhR) is a crucial transcription factor involved in remediating xenobiotic chemicals from within the human body, yet the mechanisms by which AhR is able to elicit different responses to exogenous and endogenous AhR ligands remains unclear. We hypothesize that the aryl hydrocarbon receptor nuclear translocator (ARNT), the DNA binding partner of AhR, performs a crucial role in the regulation of AhR ligand-specific gene expression. ARNT is expressed as two separate isoforms, ARNT isoform 1 and ARNT isoform 3, which differ by only 15 amino acids that make up exon 5 in ARNT isoform 1. This exon encodes a distinct phosphorylation site at serine 77 (S77), which can be induced upon exposure to the well-characterized AhR ligand and toxicant, 2,3,7,8-tetrachlorodibenzodioxin (TCDD). Upon mutation of S77 to alanine, RNA polymerase II is unable to be recruited to the CYP1A1 promoter, thus leading to a decrease in CYP1A1 transcription and leading to the importance of ARNT isoform 1 phosphorylation in AhR signaling. Differing functions of the ARNT isoforms have also been identified in AhR signaling through next generation sequencing, molecular techniques, and mass spectrometry in a human T cell lymphoma cell line. Uniquely recruited transcriptional co-regulators were found bound to ARNT isoform 1 or ARNT isoform 3 following mass spectrometry analysis with and without TCDD treatment, thus indicating another possible role the ARNT isoforms might perform in AhR signaling and suggesting that ARNT isoform 1 phosphorylation is required for the recruitment of unique co-regulators and activation of AhR target genes. Upon further validation of these co-regulator findings, we have continued exploration of the proteins interlink enhancer binding factor 3 (ILF3) and tripartite motif containing 28 (TRIM28) and the role they perform in AhR-ARNT signaling. These data indicate the critical roles of the ARNT isoforms in AhR signaling.

**3026 Regulation of ARNT Alternative Splicing**

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The aryl hydrocarbon receptor (AhR) is a cytosolic receptor that mediates the effects of environmental contaminants on the body. When activated, AhR translocates to the nucleus and dimerizes with the aryl hydrocarbon receptor nuclear translocator (ARNT) and together they bind to xenobiotic response elements of ARNT-related genes. During normal physiology this receptor plays a role in the differentiation of various pathways including hematopoiesis, B and T cells, and lymphoid systems. Toxic responses can occur when AhR is activated by exogenous ligands, like benzo-a-pyrene, and aberrant activation can occur. The aryl hydrocarbon receptor nuclear translocator (ARNT) is alternatively spliced to produce two isoforms, ARNT isoform 1 and 3. These isoforms differ in just 15 amino acids present in exon 5 that is included in the final mRNA transcript of ARNT isoform 1. Interestingly, normal lymphocytes have equal amounts of ARNT isoform 1 and 3, but lymphoid malignancies have ARNT isoform 3, which differ by only 15 amino acids that make up exon 5 in ARNT isoform 1. This exon encodes a distinct phosphorylation site at serine 77 (S77), which can be induced upon exposure to the well-characterized AhR ligand and toxicant, 2,3,7,8-tetrachlorodibenzodioxin (TCDD). Upon mutation of S77 to alanine, RNA polymerase II is unable to be recruited to the CYP1A1 promoter, thus leading to a decrease in CYP1A1 transcription and leading to the importance of ARNT isoform 1 phosphorylation in AhR signaling. Differing functions of the ARNT isoforms have also been identified in AhR signaling through next generation sequencing, molecular techniques, and mass spectrometry in a human T cell lymphoma cell line. Uniquely recruited transcriptional co-regulators were found bound to ARNT isoform 1 or ARNT isoform 3 following mass spectrometry analysis with and without TCDD treatment, thus indicating another possible role the ARNT isoforms might perform in AhR signaling and suggesting that ARNT isoform 1 phosphorylation is required for the recruitment of unique co-regulators and activation of AhR target genes. Upon further validation of these co-regulator findings, we have continued exploration of the proteins interlink enhancer binding factor 3 (ILF3) and tripartite motif containing 28 (TRIM28) and the role they perform in AhR-ARNT signaling. These data indicate the critical roles of the ARNT isoforms in AhR signaling.
**3027 Coffee as an Aryl Hydrocarbon Receptor (AhR) Agonist**

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Coffee is one of the most popular beverages worldwide, and several studies show a correlation between increased daily intake of coffee and decreased incidence of several conditions, including some cancers, liver and Parkinson’s disease and overall mortality. Extraction of coffee with boiling water and subsequent extraction of the water with chloroform showed that both the water and chloroform extracts induced the following aryl hydrocarbon receptor (AhR)-responsive genes CYP1A1, CYP1B1 and UGT1A1 in Caco2 colon cancer cells and in a young adult mouse colonocyte (YAMC) cell line. Compared to 10 mM 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) the water and chloroform coffee extracts induced ≥25% of the TCDD-induced CYP1A1 gene expression (maximal) whereas the coffee extracts maximally induced CYP1B1 and UGT1A1. In contrast, TCDD and the coffee extracts did not induce the detoxifying enzymes in AhR-knockout cells (Caco2-AhRKO and YAMC-AhRKO) demonstrating that coffee extracts contain AhR ligands and studies show that the AhR and AhR agonist are chemoprotective against some intestinal problems. Thin layer chromatography was used to separate the coffee extracts into 3 bands, namely top, middle (containing caffeine) and bottom and subsequent bioassays of these fractions showed that the AhR activity was concentrated in the top and bottom fractions and caffeine was inactive as an AhR ligand. Analysis of the total, top and bottom extracts by gas and chromatography-mass spectrometry showed that the coffee extract contained hundreds of compounds and several of these were screened in Caco2 and YAMC cells for their AhR-dependent activity. The alkaloid norharman was identified in the coffee extract and was a weak inducer of CYP1A1 gene expression in both YAMC and Caco2 cells and a more potent inducer of CYP1B1 and UGT1A1 thus resembling the differential gene induction pattern observed for the coffee extracts. Ongoing studies are focused on investigating other active constituents in coffee and their potential beneficial effects in the intestine and other target tissues.

**3028 Aryl Hydrocarbon Receptor Activation by Para-benzoquinones Are Substitution Dependent**

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The aryl hydrocarbon receptor (AhR) is expressed in many tissues and is extensively investigated due to its activation by environmental toxins. Benzoquinones are represented widely in nature and may act as mediators of toxicity. To investigate AhR activation by compounds containing benzoquinone structures, we developed an XRE/DRE-responsive GFP reporter system designated the Aryl Hydrocarbon Receptor Activation (AHRA) assay. How structural substitutions affect benzoquinone activation of the AhR is unclear. For example, it has been hypothesized that benzoquinone containing structures may activate the AhR through alkylation, though this has not been demonstrated definitively. In the current study, para-benzoquinone activation of the AhR was found to be modified extensively by substitutions present on the para-benzoquinone moiety. Unsubstituted para-benzoquinone had relatively potent activity, whereas substitution of all unsubstituted carbons with methyl groups (i.e., duroquinone, 2,3,5,6 tetramethyl para-benzoquinone) did not activate the AhR as measured using the AHRA assay. Addition of a single methyl substitution on the para-benzoquinone structure was a more potent AhR activator than para-benzoquinone. In contrast, substitution of the para-quinone structure with 3 methyl groups greatly reduced AhR activation. Similarly, either 2,6-dimethyl- or 2,5-dimethyl-benzoquinone substitutions had reduced activity compared to methyl para-benzoquinone. Interestingly, 2,3-dimethyl benzoquinone was a relatively potent activator of the AHRA assay. Tert-butyl para-benzoquinone was found to be the most potent activator of the AHRA assay among the para-benzoquinones investigated. Results from these studies support that alkylation of electrophilic carbons may contribute to benzoquinone activation of the AhR and substitutions on the quinone moiety strongly influence the potency of AhR activation by benzonquinones.

**3029 Microbiome-Derived Aryl Hydrocarbon Receptor Ligands Ameliorate Intestinal Inflammation in Mice**

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Ligand activation of aryl hydrocarbon receptor (AhR) is an inviting therapeutic approach for inflammatory bowel disease (IBD), and dietary modulation of the gut microbiota received considerable attention. We investigated the role of microbiome-derived AhR ligands in intestinal inflammation via an integration of a mouse model of experimental colitis and a standardized black raspberry (BRB)-based dietary intervention. The results show that the BRB dietary intervention ameliorated the intestinal inflammation in mice in an AhR-dependent manner. With the dietary intervention, perturbations in the gut microbiota associated with colitis were partially restored. Furthermore, increased levels of AhR ligands in the diet-modulated gut microbiome resulted in enhanced AhR activation, which may contribute to the ameliorating effect on intestinal inflammatory status. Enriched bacterial genes and pathways in the biosynthesis of heme-related compounds that are known AhR ligands, in concert with strong correlations between specific bacterial species and these compounds suggested the involvement of gut bacterial activities in producing AhR ligands. Host metabolic profiling revealed restored metabolites that are relevant to inflammation and the gut microbiota. These findings provide insights regarding microbiome production of AhR ligands as an attractive therapeutic target of IBD via gut microbiome modulation.

**3030 PCB 126 Induces Macrophage Polarization and Inflammation through AhR and NfκB Pathways**

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Polychlorinated biphenyls (PCBs) are persistent organic pollutants that contribute to inflammatory diseases such as atherosclerosis, and macrophages play a key role in the overall inflammatory response. Depending on specific environmental stimuli, macrophages can be polarized either to pro-inflammatory (e.g., M1) or anti-inflammatory (e.g., M2) phenotypes. We hypothesize that dioxin-like PCBs can contribute to macrophage polarization associated with inflammation. To test this hypothesis human monocytes (THP-1) were differentiated to macrophages and subsequently exposed to PCB 126. Exposure to PCB 126 significantly induced the expression of inflammatory cytokines, including TNFα, IL-1β and IL-6, suggesting induction of chemokines which regulate immune cell recruitment and infiltration of monocytes/macrophages into vascular tissues. In addition, oxidative stress sensitive markers including nuclear factor (erythroid-derived 2)-like 2 (NFE2L2) and down-stream genes, such as glutathione S-transferase (GST) and NAD(P)H quinone oxidoreductase 1 (NQO1), were induced following PCB 126 exposure. Since dioxin-like PCBs can elicit inflammatory cascades through multiple mechanisms, we then pretreated macrophages with both aryl hydrocarbon receptor (AhR) and NF-κB antagonists prior to PCB 126 treatment. The NF-κB antagonist BMS-345541 significantly decreased mRNA and protein levels of multiple cytokines by approximately 50% compared to PCB treatment alone, but the AhR antagonist CH-223191 was protective to a lesser degree. Our data demonstrate the involvement of PCB 126 in macrophage polarization and inflammation, indicating another important role of dioxin-like PCBs in the pathology of atherosclerosis. These data have translational implications, suggesting that environmental insults can influence the immune system and increase inflammation, creating an advantageous environment for cardiovascular disease progression.

**3031 Methylglyoxal-Derived Advanced Glycation End Products Mediate Matrix Metalloproteinases via Activation of ERK, JNK, NF-κB Signaling Pathways**

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Advanced glycation end products (AGEs) is non-enzymatic reaction of reducing sugars and proteins. Accumulation of AGEs is associated with pathophysiological diseases, such as atherosclerosis, Alzheimer’s disease, cancer, and diabetic nephropathy. Receptor for AGEs (RAGE) mediates inflammatory signals leading to expression of inflammatory signals leading to tissue chronic cytokines. As the signal transduction RAGE is closely linked to diabetic nephropathy AGEs through its receptor RAGE have role in kidney cells inflammation proteolytic enzyme activation of Mitogen activated protein kinase (MAPK) signaling pathway. NF-κB activated range of signals including activation of MAPK path-
way process. MAPK pathway is responsible for initiating acute and chronic inflammatory in cells. In this study, we evaluated whether treatment of NRK-52E kidney cells with AGES influences the induction of matrix MMPs, which might be responsible for the development of kidney fibrotic dysfunction. The effect of methylglyoxal-derived AGES (AGE-4), among other AGES, on the induction of MMPs in NRK-52E cells was investigated. Enhanced MMP-2 and MMP-9 expression in methylglyoxal-induced NRK-52E cells was mediated by ERK, JNK, and NF-κB pathways. Therefore, our findings suggest that RAGE and AGE-4 contribute to the development of kidney fibrotic dysfunction. Effect of AGES on induction of inflammation in NRK-52E cells treated with AGES (100μg/ml). We suggest that AGES and RAGE interaction that plays an important role in MAPK pathway signals in inflammation-mediated cytokines in cells. This study indicates that AGE-4 increases MMP-2 and MMP-9 expression by RAGE expression in NRK-52E cells, and that RAGE-AGES is involved in the AGE-4 induced ERK, JNK, NF-κB signaling expression may be associated with the AGE-induced kidney dysfunction and elevated of T2DM patients via an increase in MMPs expression.

**3032 Novel Aerosol Treatment of Airway Hyper-Reactivity and Inflammation in a Murine Model of Asthma with an Inhibitor of Soluble Epoxide Hydrolase**

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Asthma affects more than 300,000,000 people in the world today, and the number of patients is increasing. Multiple methods of therapeutic intervention have been proposed, including steroid-based treatment modalities. Steroid-based treatments weaken overactive immune responses and control inflammation. However, they also carry some side effect risks to the immune system, including adverse effects on the liver, kidney dysfunction, and elevated of T2DM patients via an increase in MMPs expression. We discovered that NM treatment decreased MCP expression with specific small-molecule inhibitors. In previous studies, we and others found that skin inflammation effects induced by PGD2-G can be further augmented by inactivating CES1 activity. About 50% of its hydrolytic metabolism, and that PGD2-G could be stabilized by the inclusion of CES1 inhibitors. The inhibitor potency followed the rank order: CPO>WWL113>WWL229. THP1 macrophages co-treated with WWL113 and PGD2 prior to stimulation with lipopolysaccharide exhibited a more pronounced attenuation of pro-inflammatory cytokine levels (e.g. IL-6) than by PGD2 treatment alone. These results suggest that the anti-inflammatory effects induced by PGD2-G can be further augmented by inactivating CES1 activity with specific small-molecule inhibitors. Supported by R15GM12806-01.

**3033 Environmental Impact on Health through the Lens of Brain Microglia**


Inflammatory responses in the brain are thought to play a crucial role in the pathogenic process of a number of diseases. This inflammation may be induced by endogenous or exogenous stimuli, including neuronal death or environmental immune disruptors. The immune system plays a critical role in initiating a response to environmental stimuli, rendering the understanding of immune cell activation in the brain critical to understanding the environmental impact on the brain microglia. Microglia are resident immune cells of the brain that constantly survey their microenvironment and maintain homeostasis in the brain. This function of microglia suggests that these cells may be critical to understanding susceptibility to environmentally induced neurotoxicity and central nervous system disease pathogenesis. How the brain perceives the environment and whether it disseminates effects on the rest of the body when it is under attack has remained poorly understood. This study reveals a multi-organ immune response that is influenced by immune cell activation in the brain and results in dramatic events in peripheral tissues. Specifically, utilizing zebrafish larvae as an experimental model, we show that lipopolysaccharide (LPS)-induced neuroinflammation results in macrophage accumulation in the periphery in a tissue-specific and circulation-dependent manner. Additionally, we detect a systemic and tissue-specific upregulation of an acute-phase response gene, serum amyloid A (SAA), and a tissue-specific cytokine gene, metalloproteinase (MMP)-14 and inducible nitric oxide (iNOS) expression, suggesting a role for the p3023-stat3 signaling pathway in mediating a peripheral body response to brain inflammation. This study reveals dynamic functions of macrophages that may uncover how brain-specific perturbations might have widespread impacts on the rest of the body, and how changes to macrophage behaviors may provide a means to assess tissue-specific response to toxic stimuli.

**3034 Inactivation of Prostaglandin D2, Glycerol Ester Hydrolysis Using Carboxylesterase 1 Inhibitors Augments Its Anti-inflammatory Effects in Human THP1 Monocytic/Macrophage Cells**

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Human mononuclear cells in blood have important roles in host defense and express the enzyme carboxylesterase 1 (CES1). This metabolic serine hydrolase plays a critical role in the metabolism of many molecules, including prostaglandins called eicosanoids and oxygenated (PG) which are formed during cyclooxygenase-mediated oxygenation of the endocannabinoid-2-arachidonoylglycerol. Some PG-Gs have been shown to exhibit anti-inflammatory effects. However, they are unstable compounds and their hydrolytic breakdown generates pro-inflammatory prostaglandins. We hypothesized that by blocking the ability of CES1 in monocytes/macrophages to hydrolyze PGs, the beneficial effects of anti-inflammatory PGD2-G could be augmented. The goal of this study was to determine whether PGD2-G is catalyzed by CES1, than to evaluate the degree to which this metabolism is blocked by small-molecule inhibitors. A human monocytic cell line (THP1 cells) was pretreated with increasing concentrations of small-molecule inhibitors that block CES1 activity (chlorpyrifos oxon (CPO), WWL229, or WWL113), followed by incubation with PGD2-G (10 μM). Organic solvent extracts of the treated cells were prepared and analyzed by LC-MS/MS to assess levels of the hydrolysis product PGD2. Further, THP1 monocytes with normal CES1 expression (control cells) and knock-down CES1 expression (CES1KD cells) were employed to confirm CES1’s role in PGD2-G catalysis. We found that CES1 has a prominent role in PGD2-G hydrolysis in this cell line, accounting for about 50% of its hydrolytic metabolism, and that PGD2-G could be stabilized by the inclusion of CES1 inhibitors. The inhibitor potency followed the rank order: CPO>WWL113>WWL229. THP1 macrophages co-treated with WWL113 and PGD2 prior to stimulation with lipopolysaccharide exhibited a more pronounced attenuation of pro-inflammatory cytokine levels (e.g. IL-6) than by PGD2 treatment alone. These results suggest that the anti-inflammatory effects induced by PGD2-G can be further augmented by inactivating CES1 activity with specific small-molecule inhibitors. Supported by R15GM12806-01.

**3035 Skin Inflammation Following Exposure to Nitrogen Mustard Alters Levels Epigenetic Chromatin Modification Enzymes**

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Inflammatory signaling networks are critical for oxidant defense and redox surveillance. However, many of the molecular events in cellular signaling following the exposure of skin to mustard agents remain poorly defined. Nitrogen mustard (bis-[2-chloroethyl]methylamine; NM) is a chemical threat agent that can cause inflammation, edema and ulcerative wounds in the skin. Currently there are no approved medical countermeasures to reverse the effects of NM. In previous studies we and others found that skin inflammation following exposure to NM is driven at least in part by oxidative stress. In the present studies, we tested the hypothesis that NM derived skin inflammation involves the modification of specific epigenetic pathways known to be modulated by cellular redox status. Using transcriptomics profiling and PCR gene array analysis, we visualized the effects of NM mouse keratinocyte PAM 212 cells and CD-1 mouse skin. We found that NM decreased DNA methyltransferase, histone methyltransferase and associated SET domain protein levels in PAM 212 cells. We hypothesized that these effects could regulate histone methyltransferase activity. We discovered that NM treatment decreased levels of Histone acetyltransferase 1 (K-specific demethylase, and Histone Acetyltransferase and Nuclear receptor coactivator 3 (Ncoa3) mRNA.
Interestingly, these alterations were associated with decreases in both histone phosphorylation and histone ubiquitination. Furthermore, we identified similar patterns of mRNA levels for histone methyltransferases, histone phosphorylation and histone ubiquitination modulating enzymes in NM-treated CD-1 mouse epidermis. Taken together, our transcriptomic profiles revealed markedly similar patterns of epigenetic chromatin modification enzymes in murine skin and keratinocyte cells. We speculate that these patterns play a critical role in regulation of inflammatory signaling in response to exposure to NM. Supported by NIH AR055073.

3036 Carbon Nanotubes Promotes Atherosclerosis Due to Enhancing Inflammatory Responses Triggered by Inflammasome Activation


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Asbestos and silicon have been shown to activate the NLRP3 inflammasome. Inflammasome activation is triggered by reactive oxygen species, which are generated by a NADPH oxidase. Carbon nanotubes are a valuable industrial product but there is potential for human pulmonary exposure during production and their fibrous shape raises the possibility that they may cause inflammation and oxidative stress. We hypothesized that carbon nanotubes promote atherosclerosis due to enhancing inflammatory responses triggered by inflammasome activation. The present study investigated the roles of NLRP3 inflammasome in the atherosclerosis lesion induced by carbon nanotubes (DWCNTs) using apolipoprotein E-deficient (ApoE−/−) mice, a model of human atherosclerosis. Male ApoE−/− and age-matched wild type (WT) mice (n = 8 for each group) were treated with short or long DWCNTs (10 or 40 μg/mouse) once every other week for 8 weeks by pharyngeal aspiration.

After treatment, the aortic arch was stained with oil red-O solution to evaluate the extent of atheroma formation. ApoE−/− mice exposed to CNTs at the high dose showed increased plaque area in the aorta by oil red-O staining. RNA was extracted from the thoracic aorta and the expression of inflammasome activation was analyzed. The expression of NLRP3, which is a central constitutive factor of inflammasome, and the expression of inflammatory factors (VCAM-1, ICAM-1) and cytokine (MCP-1) were significantly increased in ApoE−/− mice treated with long DWCNTs at the high dose. Furthermore, in human umbilical vein endothelial cells (HUVECs) treated with the high dose of DWCNTs the level of cytokine (IL-1β) was significantly elevated. The results suggested that DWCNTs induced the formation of arteriosclerotic lesion due to enhancing inflammatory responses triggered by inflammasome activation.

3037 Effects of Deficiency of FXR and Lcn2 on Liver Injury in a High-Fat Diet-Induced Mouse NAFLD Model

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Farnesoid X receptor (FXR), well-known for maintaining bile acid homeostasis, also critically regulates liver inflammation. FXR expression is downregulated with inflammation and in patients with non-alcoholic steatohepatitis (NASH). NASH is an aggressive form of non-alcoholic fatty liver disease (NAFLD) characterized by steatosis, lobular inflammation, hepatocyte ballooning, and even fibrosis. About 30% of adults in the U.S. have NAFLD, with 5-10% estimated to progress to NASH, potentially leading to irreversible cirrhosis and hepatocellular carcinoma. FXR has been shown to regulate innate immunity and one of the mechanisms is by inducing acute phase proteins (APPs) in hepatocytes. APPs are increased in response to acute phase response (APR), a systemic reaction to tissue injury or infection. Lipocalin 2 (Lcn2) is an APP that is responsive to cellular stress (cell injury or inflammation) and overexpressed in livers of NASH patients. This study aims to assess NAFLD development in mice deficient in both hepatic FXR and Lcn2. Wild type (WT), FXR−/−/Lcn2−/−, Lcn2−/−/FXR−/− (DKO) mice were fed a control (CD, 10% kcal) or high-fat diet (HFD, 60% kcal) for 1, 3, and 6 months. Genotypes were characterized for phenotypic differences based on serum markers, liver gene expression levels, and histology. DKO-HFD mice had worsened liver injury compared to all other genotypes by 6 months, with worsened steatosis and liver inflammation via H&E staining and elevated liver weight (9.7%). DKO-HFD mice had elevated serum ALT (290 U/L), total bile acid (TBA, 94 μmol/L), and cholesterol levels (297 mg/dL). Mcp-1 mRNA levels were significantly increased 10-fold by 3 months in DKO-HFD mice compared to all other genotypes, and this induction was sustained, while Lcn2−/−/FXR−/− mice achieved 10-fold induction by 6 months. Hepatic FXR mRNA was negatively correlated to TBA, ALT, Mcp-1, and Lcn2 levels, and hepatic Lcn2 mRNA was positively correlated to ALT and TBA levels. FXR and Lcn2 deletion worsened NASH phenotype; therefore, we propose that FXR, the APR, and Lcn2 play a significant role in preventing NASH progression.

3038 Voltage-Gated Kv1.3 Activation and Expression Are Increased in Neurotoxic Models of Parkinson’s Disease

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Chronic neuroinflammation sustained by persistent activation of the microglial cells is a primary contributor to the neurodegenerative processes of Parkinson’s disease (PD). Identification of key targets that regulate neuroinflammation will help in the development of novel therapeutic agents for the treatment of PD. Herein, we demonstrate that expression of the voltage-gated potassium channel Kv1.3 is significantly increased in microglia activated by misfolded aggregated α-synuclein (αSyNagg). The upregulated expression of Kv1.3 was also observed in various PD paradigms, including the neurotoxic MPTP model and the transgenic MitoPark model, and was further verified in postmortem PD patients. Notably, patch-clamping of Kv1.3 on microglia revealed that the increased expression levels were positively correlated with increased channel activity. The increase in expression and activity levels occurred concomitantly with the production of proinflammatory cytokines, suggesting that Kv1.3 regulates inflammatory inflammation. Indeed, pharmacological inhibition of Kv1.3 by PAP-1 significantly attenuated the production of proinflammatory cytokines in αSyNagg-stimulated microglia. Furthermore, oral administration of PAP-1 exhibited neuroprotection against MPTP-induced dopaminergic neurotoxicity and neuroinflammation. The Kv1.3 inhibitor also effectively reversed the motor deficits and the loss of striatal dopamine content in the chronic, progressively degenerative MitoPark mouse model of PD. Collectively, we demonstrate that Kv1.3 is vital for microglia-driven inflammation, contributes to dopaminergic neurotoxicity, and is a potential therapeutic for PD.

3039 Effects of Different Liquid Flow Rates on Cytotoxicity and IL-8 Secretion Level of Human Lung Macrophages


The human lung macrophages are the innate immunity, play a key role in the COPD’s progression. As its populations are mainly reside at the airspaces of the lung and localise in the interstitial space between the alveoli and blood vessels, macrophages are exposed to mechanical stress during the respiratory movement. Studies have shown that the mechanical stress generated by mechanical ventilation is the cause of further inflammatory damage in COPD patients. However, macrophages are typically cultured in a resting medium in vitro, which does not mimic the mechanical stress that macrophages undergo in vivo. The objective of this study was to use a drive-buffer-circulation system to generate a certain liquid flow rate to form mechanical stress to simulate the mechanical environment in which macrophages were exposed in vivo and to evaluate the effects of different flow rates on cytotoxicity and IL-8 secretion level of human lung macrophages. Human lung macrophages were cultured in the system (cell suspensions were distributed in buffer chamber (35 mL) and drive-circulation pipes (5 mL), respectively) and exposed to different liquid flow rates (0, 1, 5, 10 mL/min) for 3h. At 0, 0.5, 1, 2 and 3h time points, 0.8 mL cell suspensions in the system were separately removed for real-time detection or resting culture for 24 h before detection. CCK-8 proliferation assay was used to detect the cytotoxicity and ELISA test was used to detect IL-8 secretion levels. Compared with the 0 mL/min (0, 0.5, 1, 2 and 3h) groups, the results showed that the mechanical stress produced by the system decreased the survival rate of macrophages and promoted the secretion of IL-8 in a flow rate- and time-dependent manner, and the 1 mL/min (0, 0.5, 1, 2 and 3h) groups had no significant effect on survival rate of macrophages and IL-8 secretion levels. In addition, the resting culture 24h groups had lower cell viability and IL-8 secretion levels than the real-time test groups. These results suggest that in this system, mechanical stress which generated by liquid flow rates greater than 1 mL/min may induce macrophages cytotoxicity and inflammatory responses; and liquid flow rates less than 1 mL/min can be further used to study the mechanical environment in which macrophages are under in normal bodies.
A chronic disease condition of the gastrointestinal tract of humans, inflammatory bowel disease (IBD) is manifested as severe diarrhea, fatigue, and weight loss. Factors including genetic susceptibility, mucosal immune dysregulation, gut dysbiosis and exposure to toxic metabolites of drugs and chemicals play a role in the pathogenesis of IBD. Current treatment regime is predominantly symptomatic and provides limited relief. Due to inherent anti-inflammatory and immune modulatory effect, the use of cheese cultures could be a potential strategy against IBD. In this study investigated the anti-IBD effect of two cheese culture strains using a cell culture and mice model. Two cheese cultures Lactococcus lactis ssp lactis (MS8) and Streptococcus thermophilus (TA 61) were used in this study. These cultures were grown at 37 °C in deMan, Rogosa Sharpe broth for 24 h. Caco-2 cells were cultured in DMEM with 20% fetal bovine serum at 37°C for 16 - 20 days. Following differentiation, the monolayers were exposed to cheese cultures (~ 7 log CFU/mL) for 24 h. The cells were then treated with a cytokine cocktail (IL-1β, TNF-α, IFN-γ and LPS) for 24 h, to activate maximal inflammatory response. Inflammatory response was assayed by estimation of IL-8, pNF-κB and transcellular electrical resistance (TEER). The study was replicated thrice with duplicate samples, and the data were analyzed using Proc mixed procedure of SAS 9.4. In the mice study, IBD was induced with 2% dextran sodium sulphate administered orally on the fourth week following dietary supplementation of cheese cultures. Effects of the treatment were assessed by measuring the disease activity index (DAI), intestinal permeability and colonic inflammation. Exposure to probiotics prior to cytokine treatment significantly reduced the activation and nuclear translocation of NF-κB compared to cytokine control (P <0.05). Further, the reduction in pH in the stomach was associated with a significant reduction in pro-inflammatory cytokine production namely, IL-8 (P<0.05) and decreased intestinal permeability. The mice study validated the protective effect of cheese cultures in IBD by decreasing inflammation, reducing the DAI and improving barrier integrity. Results of our study indicate that the cheese starter cultures could be employed in the management of IBD.
The blood coagulation factor fibrinogen can modulate inflammation by altering leukocyte migration and activity. To date, analyses of fibrinogen-mediated pro-inflammatory activity have largely focused on leukocyte integrin binding activity revealed by conversion of fibrinogen to a fibrin polymer by blood coagulation enzymes. Entirely separate from clot formation, fibrinogen can serve as a substrate for tissue transglutaminase (TGM2), a widely-expressed enzyme involved in tissue injury. We tested the hypothesis that the pro-inflammatory activity of surface-adered fibrinogen is fundamentally altered through cross-linking by TGM2. Mouse bone marrow-derived macrophages (BMDMs) were cultured on tissue culture plates coated with fibrinogen or TGM2-cross-linked fibrinogen (10 μg/mL) and then stimulated with lipopolysaccharide (LPS, 1 ng/mL) or vehicle for various times. TGM2-mediated activity produced an atypical hybrid of fibrinogen Aα-g chain cross-linked products as determined by Western blot analyses. TGM2-cross-linked fibrinogen enhanced inflammatory gene induction (e.g., TNFα), but had no effect on expression of anti-inflammatory cytokine IL-10 relative to unmodified fibrinogen or stimulation with TGM2 alone. LPS treatment dramatically increased expression of TNFα and IL-10, and this was unaffected by unmodified fibrinogen. However, TGM2-cross-linked fibrinogen significantly reduced (>50%) LPS-mediated induction of IL-10 mRNA and IL-10 protein expression. The inhibition of IL-10 was rapid and selective, as LPS induction of TNFα was unaffected by TGM2-mediated cross-linking of fibrinogen. The results indicate that TGM2-driven cross-linking of fibrinogen selectively alters pro-inflammatory responses in macrophages and suggests a novel mechanism whereby fibrinogen may control IL-10 expression in conditions of tissue injury.
3049 Using the Aggregate Exposure and Adverse Outcome Pathways to Create a Taxonomy of Chemical Interactions Relevant to the Assessment of Human Health and Environmental Risks

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Understanding the impacts of concurrent chemical exposures on human health and the environment requires information on chemical interactions (the ability of one chemical to influence the effects of a second chemical). Chemicals interact through a variety of mechanisms. Aggregate Exposure Pathways (AEPs) organize mechanistic data on fate and transport, exposure, and dosimetry, while Adverse Outcome Pathways (AOPs) organize mechanistic data on toxicological effects. Together they provide a framework for defining causal events over the entire source-exposure-response continuum for a receptor (a person, non-human organism, or population). A taxonomy of chemical interactions is proposed that is based on the location in the continuum where a chemical interaction occurs. The taxonomy consists of a set of hierarchal categories defined using the nodes and edges of the AEP-AOP framework. Four top-level and mutually-exclusive categories are defined as: 1) interactions that occur between the source and the external exposure surface (or boundary) of an organism; 2) interactions that occur between the surface and the target site exposure; 3) interactions that occur between the molecular initiating event (MIE) and the receptor's adverse outcome (AO); and 4) interactions that only occur for population level AOs (i.e. ecological endpoints). Categories 1 and 2 have subcategories for interactions involving: transport processes, transformation processes, and direct reactions between chemicals. Category 3 has subcategories for chemicals that interact through a common MIE, a common key event on an AEP or a common AO (but with separate AOPs). Category 4 has subcategories based on direct and indirect interactions that cause an AO in a population of organisms. The categories and subcategories provide insights potentially useful in assessing the impact of chemical interactions on non-cancer risks, developing standardized definitions for interaction terms, and designing assays to predict the potential for interactions between specific chemicals. The views expressed in this presentation are those of the authors and do not reflect the views or policies of the US EPA.

3050 Exposure to a Mixture of Suspected Thyroid Disrupting Chemicals Alters T Cell Differentiation in Xenopus laevis Tadpoles

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Thyroid hormones (TH) control postembryonic vertebrate development through thyroid receptor (TR) signaling. Deficiencies in TH production during development are known to contribute to immunodeficiency in humans and poorer outcomes in autoimmune disease models. To investigate whether endocrine disrupting chemicals that act on the TH pathway can alter the development of the immune system, we tested a mixture of chemicals used in unconventional oil and gas extraction that are suspected to be TR antagonists. TH synthesis inhibitors. This mixture consists of equimass concentrations of naphthalene, ethylene glycol, ethoxylated nonylphenol, and ethoxylated octylphenol. TR antagonist activity was verified by exposing pre-metamorphic Xenopus laevis tadpoles to 1.25 nM of the TR agonist triido-L-thryonine (T3) and T3 combined with 1 μg/L or 10 μg/L of the mixture or DMSO control for two weeks and evaluated changes in T cell subsets in the thymus and spleen, using monoclonal antibodies for CD8 and CD4 (a pan T cell marker) and two-color flow cytometry. Exposure to the 10 μg/L dose of the mixture significantly increased the frequency of CD8/CD5⁺ T cells, a cell population that is analogous to mammalian CD4⁺ T cells, in both the thymus and spleen. Exposure to the mixture also increased the frequency of CD8⁺/CD5⁻ T cells in the spleen but not the thymus. Notably, the ratio of CD8⁺ T cells to putative CD4⁺ T cells in the thymus was significantly reduced by exposure to the mixture. Taken together, these data suggest that thyroid hormone disruption induced by water pollutants during pre-metamorphic tadpole development unbalances differentiation of immature T cells toward the CD4 lineage rather than CD8 lineage. Future experiments will assess whether exposure to this mixture of TH-disrupting contaminants weakens antiviral T cell responses against viral pathogens in the short-term in tadpoles and the long-term in adult frogs after metamorphosis.

3051 Issues in Modeling Continuous Endpoints for Chemical Mixtures Risk Assessment

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Dose-response modeling of toxicological endpoints expressed as continuous measures for assessing the corresponding response of a mixture of chemicals can be challenging. The issues include: 1) differences in study control response, 2) differences in chemical response extrema (max or min), 3) differences in chemical dose-response curve shape, and 4) unknown capacity of the organism to accumulate damage. For 1), control responses theoretically should be the same for the same assay system (same species, strain, sex, etc.) but sometimes differ significantly across studies, which causes the mixture result to be dependent on the choice of the reference control; there appears to be no single optimal solution to this problem, but alternatives are suggested. For 2) is the problem of statistical agonism, which chemical components can elicit a response as extreme as a full agonist. In this case, when performing dose addition, the dose representing the partial agonist response is undefined beyond its extremum and the effective total mixture dose becomes ambiguous; solutions have been published, but have limited applicability or require subjective assumptions. For 3), relative chemical potencies will not be dependent on the response level, making the standard index chemical (IC), relative potency factor approach unsuitable, as the mixture result becomes dependent on the choice of IC. There are a few solutions that have been applied, with the “harmonic mean” method being in widespread use. However, all of these solutions have limitations, particularly with low-dosed extrapolation. Issue 4) applies to the use of effect summation when independence of action has been established for some mixture chemicals. Unrestricted addition of continuous measures will eventually exceed the biological capacity for damage accumulation, or will not be consistent with the inherent kinetics of damage accumulation, neither of which will probably be known. In this case, it may be prudent to limit effect summation to the lower end of the full response range and small levels of change in the effect. Examples for all 4 issues are shown, with potential “errors” in the order of magnitude, or less, range. The views expressed here are those of the author and do not necessarily reflect the views and policies of the US EPA.

3052 Virtual Cell Simulations to Probe the Effects of Complex Mixtures from Low to Higher Concentrations

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People and ecological receptors are exposed to chemicals as mixtures, not as single chemicals. However, there still exists a great deal of uncertainty with respect to how chemicals interact at low and higher environmental concentrations. The goal of this project is to use the estrogen receptor and gene expression response as a cell signaling model to explore how chemical mixtures respond both in vitro (i.e., the absence of endogenous estrogens) and in the likely in vivo situation (where endogenous estrogens are also present). The model uses known equations from biochemistry and affinity/dissociation constants for chemicals, and estrogen receptor binding to DNA, and gene expression kinetic models to integrate knowledge. We used an agent-based system, where chemicals, the estrogen receptors, and DNA, are all different agents, to simulate how the system as a whole interacts. This allowed us to ask questions about how different amounts and levels of chemicals in our mixture, which all have different dissociation constants, lead to differences in gene expression, and to address if concentration/dose addition or response addition explain the results we are seeing. In general, we found that due to low concentration of estrogen receptor, and due to the fact that binding is largely competitive and driven by affinity, that when there are large differences in the affinity of members of a complex mixture, that the chemicals with the most affinity tend to win out, with very little interaction between members of the mixture, meaning the potency tended to reflect those chemicals.
with the most affinity. We assumed for simplicity that efficacy was the same for all chemicals, which we know is not true, and were primarily interested in changes in potency due to the mixture. Future work will expand the model to also include efficacy estimates. The US Army Chief of Engineers has approved this paper for release. The views presented in this article do not necessarily reflect current or future opinion or policy of the US Army Corps of Engineers.

**3053** A Novel Risk Assessment Model for Incorporating Co-Exposures Provides Preliminary Guideline Values for Unregulated Chemicals

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Traditional risk assessment provides guideline values based on single chemical evaluations, primarily using animal models. However, results do not account for multiple chemical exposures, may not adequately reflect human dose-response, and guideline values for internal concentrations are unavailable for many chemicals commonly detected in human biomonitoring. The Acceptable Concentration Range (ACR) model, a novel nonlinear dose-response model that incorporates co-exposures, previously found points of departure (POD) for 11 compounds below published Human Biomonitoring (HBM) or Biological Equivalent (BE) values when applied to epidemiologic data. Here, we extend the ACR model to provide PODs for 15 additional chemicals. We applied the ACR model to 26 prenatally measured compounds (µg/L) in relation to birthweight among the population-based Swedish SELMA pregnancy cohort of 1357 mother-child pairs to (1) estimate POD and asymmetric 90% CIs for these chemicals, and (2) assess how the ACR model scaled when analyzed for single compounds, chemical class groups, or a full mixture. Results: Consistent with prior analyses of 11 compounds, PODs were below published HBM/BE values for many chemicals (e.g. Bisphenol A POD =5.9 CI=2.9-16.1, vs. HBM=200). For chemicals without regulatory guideline values, PODs were generally less than the 95th% exposure level in the study population (e.g. Hexachlorobenzene POD=65.7 CI=39.2-79.4, vs. 90th%=68.9). On average, adding chemicals to the ACR model lowered estimated PODs by 24% and 34%, for the class grouping and full model, respectively, when compared to the single chemical model (e.g. Nonachlor single: POD=56.9, class grouping: POD=37.3, mixture: POD=29.3). When accounting for co-exposures in a sensitive human population, our results indicate that current regulatory guidelines may be too high. Where guideline values are unavailable, PODs can be estimated for multiple chemicals to provide empirical estimates for determining mixture assessment factors for single chemical guideline values. Future research will evaluate ACR model performance under varying assumptions and estimated POD generalizability to other study populations.

**3054** Multiple Environmental Stressors in Tropical Coastal Ecosystems Exert Biological Responses in Caenorhabditis elegans

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Cartagena Bay (CB) is one of the coastal ecosystems more influenced by industrial and domestic activities in the Caribbean. The aim of this study was to evaluate contamination patterns by inorganic and organic pollutants in CB and Great Manabi (GMSM), a Ramsar site that is the largest estuarine system on the Caribbean coast of Colombia. Sediments were sampled during both dry and rainy seasons, at twelve and three locations, at CB and GMSM, respectively. Forty seven trace elements were analyzed using ICP-MS; Hg was measured using a direct mercury analyzer; PAHs and emerging pollutants, including UV filters, fragrances, organophosphate flame-retardants, pesticides, and PCBs were quantified by GC-MSM, respectively. Analysis of PAHs and emerging pollutants in sediments was evaluated using multivariate geospatial analysis. The number of available and new chemical substances call for the development of novel in vitro methods for the detection of synergistic or cumulative effects of mixtures, since it is highly desirable to reduce animal experiments. Here, we established multiplexed mass spectrometry and targeted metabonomics assays to profile toxicologically relevant proteins. After fragmentation of cell culture samples using tryptic one peptide derived from each protein of interest is enriched by TBP antibodies which recognize a very short C-terminal epitope. The peptides can be unambiguously assigned to the proteins by tandem mass spectrometry. The target proteins are indirectly quantified by referencing the endogenous peptides to spiked synthetic isotope-coded peptide standards. HepaRG cells were chosen as a model to investigate the effects of single pesticides and combinations thereof. Based on prior global mRNA profiling experiments, we selected a subset and analyzed the 24h effect of 30

**3055** Human-Relevant Potency-Threshold (HRPT) for ERα Agonism

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The ability of chemicals to produce physiological effects depends on pharmacokinetics and potency via the modes of action (MoAs) underlying the effects. Besides pharmacokinetics, a chemical’s ability to produce a physiological effect via a particular MoA depends on its affinity for the functional components (receptors, enzymes, transporters, etc.) that comprise the MoA and the ability to alter their functional state (activity). We tested the hypothesis that dose-response, lowest effective mechanistic potency is required for chemicals to produce physiological effects by a particular MoA in humans by comparing their potencies for the ERα-agonist MoA to data from clinical trials of estrogenic effects. Systematic literature searches identified potency data for transcriptional activation of human ERα (ERTA) and for rodent uterotrophic activity (RUA) for chemicals with consistently observable, possible, and no physiologi- cal effects. Results: In a multiple regression analysis, potency data from ERTA (1E-04 CI=1E-06-1E-03), individual (17β-estradiol reference) in the form of best fit data which showed similar rank orders at 0.5 to 1E-01 the ERTA potency. Physiological estrogenic effects in clinical trials are clear for endogenous hormones and pharmaceuticals, absent for androgens, and were equivocally observed for botanical estrogens with relative ERα-agonist potencies in the range of 1.0E-03, but not lower. Based on this approach and analysis, we conservatively propose a human-relevant potency threshold (HRPT) for ERα agonism of 1E-04 relative to the potency of the endogenous estrogenic hormone 17β-estradiol or the pharmaceutical estrogen, 17α-ethinylestradiol. This approach provides a practical means for addressing hazard identification based on MoAs, for defining common assessment groups for mixtures risk assessment, and for determining candidate chemicals for AOPs. Preliminary analysis suggests future work could extend the approach to ecological species by de novo evaluations or possibly by species-extrapolation of potency data for conserved homologous molecular targets.
Development and Validation of a QuEChERS Based Gas Chromatography Tandem Mass Spectrometry Method for the Determination of Volatile Esters in Tobacco


The volatile constituents in tobacco are transferred directly from the tobacco into the mainstream of smoke without structural changes during smoking. The distinctive aroma of tobacco and specific notes in smoke are specifically related to some of these compounds. Esters usually have a sweet taste, a fruity aroma or a wine aroma, which is coordinated with the aroma of tobacco. To determine the aroma components that affect tobacco quality, 50 important esters were selected from the list of ingredients added to tobacco in the manufacture of cigarette products by major American tobacco companies, BAT and CNCTC. To satisfy the demand for monitoring esters in tobacco, a reliable and rapid gas chromatography tandem mass spectrometry (GC-MS/MS) multi-components method for the simultaneous analysis of 50 esters in tobacco was developed and validated using a QuEChERS based extraction procedure. Tobacco samples were soaked in acid solution to adjust to a pH of 3, extracted with acetonitrile and cleaned up by SinChERS-Nano purification column. Multiple reaction monitoring mode was applied for the quantification and confirmation of those compounds. The calibration curve of 50 esters presented good linearity. Average recoveries of all of the compounds in tobacco were in the range of 60-158% with relative standard deviations of 0.74-18.82% at three fortification levels. The limits of quantification (LOQs) of 38 compounds ranged from 0.002 to 0.087 μg/g at the signal-to-noise ratio (S/N) of 10, and the LOQs of the remaining 12 compounds is in the range of 0.10-0.46 μg/g. The results indicated that GC-MS/MS exhibits better performance in sensitivity and throughput comparing to GC-MS. The validated method was successfully applied to the analysis of real tobacco samples, Methyl hexadecanoate had the highest detected frequency, and this was followed by Methyl octadecanoate, ethyl palmitate and Methyl oleate. These results demonstrated that the method could be applied to the analysis of esters in tobacco samples.

An Organotin Mixture Inhibits Tributyltin's Adipogenic Differentiation in 3T3-L1 Cells

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Organotin (OT) chemicals (butyltins and phenyltins) are used as stabilizers in pipes and foams. Tributyltin (TBT), and more recently dibutyltin (DBT), and triphenyltin (TPT) have been shown to stimulate adipogenic differentiation. Although these chemicals coexist in the environment, their effect in combination remains unknown. We investigated the adipogenic effect of eight OT chemicals upon either a single exposure or as a mixture using the preadipocyte 3T3-L1 cell line and hypothesized that OT mixtures would exacerbate the adipogenic effect of single OT exposures. TBT, added to adipogenic medium for 2 days followed by 8 days of growth medium plus insulin (1μg/ml). Cells were exposed to the vehicle (DMSO), dexamethasone (positive control), or a single OT (at 10 or 50 ng/ml; monobutyltin (MBT), DBT, TBT, tetrabutyltin (TebT), monophenyltin (MPT), diphenyltin (DPT), TPT, or tin chloride (SnCl2)). After 10 days, body lipid was used for lipid droplet identification as a marker of adipogenic differentiation and cells were harvested to measure mRNA expression of markers of late adipogenesis, such as FABP4. OTs that stimulated adipogenesis (DBT, TBT, and TPT) were used in dual or triple mixture combinations. Cell viability by MTT assay was only reduced in TBT50-DPT50-TPT50 (P<0.05). The strongest adipogenic differentiation occurred with TBT exposure (in a dose-dependent manner) followed by TPT and DBT (P<0.05). Adipogenic differentiation in a dual combination of TBT10 with DBT10 or TPT10 was similar to TBT10 single exposure but was reduced in dual exposure with DBT50 or TPT50 (P<0.05). TBT50-induced differentiation was also reduced in dual mixture with TPT10, TPT50, or DBT50 (P<0.05), but not DBT10. Both triple mixtures resulted in reversal of TBT’s adipogenic differentiation down to control levels (P<0.05). TBT-, DBT-, or TPT-treated cells (both 10 and 50 ng/ml doses) displayed a dose-dependent upregulation of FABP4 (P<0.05). All dual and triple combinations of TBT, DBT and/or TPT had upregulated expression of the control group (P<0.05). We demonstrated that DBT and TPT could counteract TBT’s adipogenic differentiation. These findings highlight the potential antagonistic effects among OTs at environmentally relevant doses and the need to understand the effects and mechanism of action of complex OT mixtures on adipogenic outcomes. Funding: NIEMS/NIMH Z2ES026208 to A.V.L.

Predicting the Activation of the Androgen Receptor by Mixtures of Ligands Using Generalized Concentration Addition

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Assessing the health effects of chemical mixtures is a crucial and difficult challenge. Concentration addition/dose addition (CA) is widely used for compounds that act by similar mechanisms. But CA cannot make predictions for mixtures that contain full and partial agonists for effect levels above that of the least efficacious component. As partial agonists are common, we developed Generalized Concentration Addition (GCA). Based on mechanistic information, we use pharmacologically-based Monte Carlo simulations to estimate the biological effect of mixtures; we test the predictions with empirical data. GCA has been successfully applied to systems where ligands compete for a single binding site: AhR ligands and PPARy ligands. Here, we develop a pharmacodynamic model for a system with two binding sites, the androgen receptor (AR). AR acts according to the classic homodimer activation model: each AR protein in the cytoplasm binds ligand, undergoes a conformational change that relieves inhibition of dimerization, and binds to DNA response elements as a dimer. The model meets the mathematical requirements for GCA.
iodine precursors that may enhance the formation of iodinated DBPs (I-DBPs). In the current study, source water containing one of the four ICM, iopamidol (IPAM), iohexol, iopromide, or diatrizoate, which are the ones detected most frequently in water, was chlorinated; non-chlorinated ICM-containing water samples served as controls. We quantified 21 regulated and non-regulated DBPs, 11 target I-DBPs, and performed a non-targeted, comprehensive, broad-screen identification of I-DBP formation. Mutagenicity of XAD-resin extracts of non-chlorinated and chlorinated ICM-containing water was examined in Salmonella strains with and without metabolic activation. ICM alone, i.e., without chlorination, did not result in DBP formation. However, the presence of IPAM increased chlorine demand and enhanced formation of bromoacetic acid, trichloroacetic acid, dichloroacetonitrile, dichloroacetonitrile, and trichloroacetaldehyde. IPAM also enhanced the formation of I-DBPs, notably dichlorodimethane, chlorodioxidemethane, iododioxetadie, iodooxetic acid, and chloriodoiodestic acid. Non-targeted, comprehensive, broad-screen analysis identified novel I-DBPs, including 1 new iodomethane, 3 new iodooxetic acids, and 2 new iododioxetanes. Mutagenicity of chlorinated source water was not increased by addition of any of the 4 tested ICM, regardless of metabolic activation status. In summary, DBP concentrations, including I-DBPs, were increased in extracts of chlorinated IPAM-containing source water, but Salmonella mutagenicity was not. This abstract does not reflect US EPA policy.

3063 Validation of a Screening Platform for Vascular Toxicities Utilizing Synthetic Substrates

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Angiogenesis is an essential developmental process that remains critical throughout the lifespan in response to conditions requiring increased oxygen supply, including physiological repair and exercise. The process is complex and tightly regulated through localized delivery of angiogenic factors, matrix remodeling, cellular migration and vessel maturation. The angiogenic process is also a critical component of several disease pathologies including cancer, neuropathies, critical limb ischemia, muscular degeneration and vascular malformations. For several decades, a primary in vitro assay to assess for vascular disrupters has relied on animal-derived biomaterials such as the Englebreth-Holm-Swarm mouse sarcoma-derived products marketed as Matrigel®, Gelnox® and Cultrex®. This material is complex, exhibits lot-to-lot variability and is challenging in an HTS workflow due to its temperature sensitivity. In collaboration with scientists at the University of Wisconsin, we have developed a synthetic vascular hydrogel that enables high throughput screening (HTS) for vascular disruptors utilizing human umbilical vein endothelial cells (HUVEC) or IPSC-derived endothelial cells. The hydrogel is optimized to promote a VEGF-dependent tubulogenic response which requires matrix remodeling and cellular migration. The assay detects appropriate inhibitors of these processes including Axitinib (AG013736) (IC_{50} 300 nM), Sunitinib (SU12148) (IC_{50} 6.3 µM), Nocodazole (IC_{50} 23 nM) and Pristomast HCl (AG3340 hydrochloride) (IC_{50}< 1 µM). The assay can be run in a 96 or 384 well plate format with Z' values of >0.5 and is insensitive to Suramin (Bayer 205), a compound which disrupts Matrigel and is a broadly referenced false positive in the Matrigel-based assay. Overall, the hydrogel platform is flexible for use in standard cell culture workflows and is suitable for co-culture and 3D organoid applications, facilitating their use for toxicity or efficacy screening applications. (1) Nguyen, E. H., W. T. Daly, N. T. N. Le, E. Farnooshian, D. G. Belair, M. P. Schwartz, C. S. Lebakken, G. E. Ananian, M. A. Saghir, T. R. Knudsen, N. Shelbain and W. L. Murphy. Versatile synthetic alternatives to Matrigel for vascular toxicity screening and stem cell expansion. Nat Biomed Eng 1, (2017).
tissue growth by day 4; 2) epithelial cell morphology similar to human colon; 3) a physiological TEER value of >300 Ω·cm² mimicking the colon microenvironment; and 4) expression of CK19 (epithelial cell marker), vimentin (fibroblast cell marker), and Alicant blue PAS staining (mucous producing goblet cell marker) on the villi-like structure. When mixed epithelial cells and fibroblasts are seeded on tissue culture inserts, there was a self-assembly pattern of differentiation with the fibroblasts occupying the base layer and the epithelial cells differentiating and stratifying on the apical layer. This new human cell-based colon tissue model will be a useful tool for pre-clinical assessment of microbiomes, mucosal inflammation, and screening of colorectal cancer products for their irritant potential. Such models will also reduce the use of animals for experimentation.

**3065 High-Throughput and Physiologically- Relevant Anisotropic hiPSC-Derived Cardiomyocyte Cultures Provide Better Resolution over Safety Profiles of Compounds with Known Cardiotoxic Mechanisms of Action**


Drug removal from the clinical market, as well as late-stage failures in clinical trials, are often linked to unforeseen cardiac toxicity. hiPSC-CMs are an integral component of a new paradigm, the Comprehensive in vitro Proarrhythmia Assay (CiPA) Initiative, through which panels of compounds with known mechanism of cardiotoxicity are being evaluated on hiPSC-CM platforms across independent test sites and through cutting-edge technologies. Key challenges under consideration for the hiPSC-CM system are sub-ideal cardiomyocyte geometry, sub-cellular structural organization, and electro-physiological maturity. Bioengineering approaches developed to enhance hiPSC-CM maturity have shown improvements in aspects of hiPSC-CM physiology, however those approaches have limited scalability and thus are not amenable to high throughput screening. hiPSC-CMs cultures plated on a high throughput platform which passively promote cardiomyocyte alignment have been shown to display physiologically-relevant features, including more physiologically cellular geometry, coherent unidirectional contraction, cardiac cell junction re-modeling, and improved calcium handling. To evaluate whether the changes induced by this platform translated into differential responses to cardio-active compounds, high throughput calcium flux assays were performed on hiPSC-CMs cultured in standard high throughput screening cell cultureware or anisotropic 384-well plates and subsequently interrogated with the 28 compounds included in the CiPA initiative. Interestingly, when combining high and intermediate risk compounds, differential responses were observed in 63% of the compounds tested. Specifically, all compounds in the high risk category showed a clearer dose-dependent progression in the severity of early afterdepolarizations (EADs). Six out of eleven compounds in the intermediate risk category, namely Pimozide, Droperidol, Cisapride, Astemizole, Domperidone and Terfanadine, showed a more sensitive response in anisotropy. No EADs were observed in either control or anisotropic conditions treated with low risk compounds. Altogether, anisotropic high throughput hiPSC-CM cultures formatted in the platform employed in this study showed better resolution over the progression and severity of pro-arrhythmic events.

**3066 Whole Transcriptome Extrapolation and Mechanism of Action Analysis Using GENIE Pipeline**


The Tox21 consortium is tasked to identify patterns of chemically-induced biological responses in order to characterize toxicity and disease pathways in a high-throughput manner and prioritize compounds for more extensive toxicological evaluations. The Tox21 is pursuing alternatives to cost prohibitive gene expression assessment techniques (Microarray or RNA-seq) that enable assessment of selected transcriptomic subsets in a high-throughput manner. Subsets measured using those techniques are not readily usable with standard differential pathway detection algorithms like Gene Set Enrichment Analysis (GSEA). This can yield limited gene-level differential expression results, necessitating the extrapolation of unmeasured portions of the transcriptome using inferences from measured portions. This can be achieved by modeling gene to gene interconnectedness of the transcriptome via large curated training samples with available whole transcriptome measurements. We present results of a transcriptome extrapolation comparing two popular large data extraction techniques: principal component regression (PCR) and Deep Learning (DL). For PCR, an eigenvalue ratio threshold of 0.1 was applied to identify significant PCs and perform prior standardization of inputs. For DL, a multi-task multi-layer feedforward neural network consisting of one input layer, 3 hidden layers (with 3,000 hidden units each), and one output layer was customized. RMA normalized signal for all unique (N=117,559) GPL570 microarray data files from NCBI Gene Expression Omnibus (GEO) were downloaded and computed. The probe-set signal values were averaged to compute gene-level signal over NCBI’s gene information database. The signal from 2,729 NCBI genes was used to extrapolate signal for 18,167 genes using each method. The extrapolation performance of each method was evaluated using 20-fold cross validation. Results indicate that PCR extrapolation outperforms DL extrapolation in terms of root mean square error (RMSE; PCR=0.39 vs. DL=0.51), median absolute error (MAE; PCR=0.20 vs. DL=0.26), and rank-biased overlap between the top 10% of true and extrapolated genes (RBO; PCR=66% vs. DL=58%). Additionally, PCR extrapolation is less computationally intensive, increasing its overall utility. These extrapolation efforts improve the scale and utility of transcriptomic data to fill in gaps between subsets of genes measured in individual studies and the ability to assess the entire transcriptome.

**3067 Improving Efficiency in Systematic Reviews by Automated Data Extraction: A Case Study Using NTP’s SRIE Challenge Dataset**

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Systematic reviews are labor-intensive exercises, especially the data extraction step 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 upstream literature screening and prioritization steps in systematic reviews, the use of information extraction algorithms in data extraction is still nascent, potentially owing to the lack of publicly available annotated datasets. In July 2018, the National Toxicology Program (NTP) conducted a public challenge, in which our group participated, to develop and apply information extraction algorithms to an annotated dataset of 100 articles related to toxicity. The articles were annotated with respect to commonly extracted data elements such as species, strain, dose, dose units, dose duration, endpoints, sample size, test article, and vehicle. In this research, we leverage NTP’s publicly accessible training dataset to test the accuracy and performance of various cutting edge information extraction algorithms. (The NTP’s test datasets were not publicly available at the time of performing this research, nor were the results of the challenge). Specifically, we split the NTP’s publicly available training dataset of 100 annotated articles randomly into a set of 80 articles used for model building (our training dataset) and a set of 20 articles used for validating results (our validation dataset). We fit a pipeline of information extraction algorithms including conditional random fields (CRF) models, long short-term model (LSTM) networks, and dictionary-based approaches to annotate the text in the validation dataset. We compared our predicted annotations with NTP’s gold standard annotations using NTP’s publicly available evaluation software that was custom developed for this challenge. For the various data elements predicted, we found extraction recall (sensitivity) rates ranging from 23% to 87%, with F1-scores ranging from 16% to 63%. We assess reasons for the differing levels of performance across various data elements, including challenges with the annotation framework and issues with the detection of phrases. We also compare the performance of the individual algorithms and their performance in sequence to assess their relative strengths. We estimate potential time savings from the use of this technology and, based on our empirical findings, propose a framework to minimize relevant data losses and maximize efficiency.

**3068 Using Liquid Chromatography Mass Spectrometry (LC-MS) to Assess the Effect of Age, Diet, and Rat Strain on the Global Metabolome**


The exposome encompasses the entire environmental exposures of an individual during a lifetime. These exposures include diet, lifestyle, environmental toxins, and workplace exposures. The interactions of combined exposures can lead to exacerbation of disease. The goal of this study was to use liquid chromatography mass spectrometry (LC-MS) to assess metabolic changes in three distinct animal strains based on two different diets. Sprague-Dawley (SD), Fischer 344 (F344), and Brown-Norway (BN) male rats were maintained on a high fat, Western (HF), or regular diet for 24 weeks. Serum was collected at 4, 12, and 24 weeks to assess metabolite changes. A cold methanol biopsy from the liver was then collected at 4, 12, and 24 weeks to assess metabolite changes. A cold methanol biopsy from the liver was then collected at 4, 12, and 24 weeks to assess metabolite changes.
3069 Physicochemical Characterization and In Vitro Toxicity of Emissions from a 3D Printer


Three dimensional (3D) printers are widely used for prototyping and building small physical objects in schools, home and businesses. Feedstock material used in 3D printing is polymer thermoplastic filament that may contain additives such as metals, ceramics, wood fiber, carbon fiber, graphene, or silica to impart aesthetic or functional properties. The use of 3D printers with polymer thermoplastics is of concern for workers and consumers because they emit a mixture of ultrafine particles and volatile organic compounds (VOCs) that are associated with respiratory and cardiovascular diseases. The scope of this study was to characterize aerosolized emissions from 3D printers and evaluate their toxic effects in human small airway epithelial cells (SAEC). Emissions were generated from a commercially available 3D printer while operating for 1.5 h with acrylonitrile butadiene styrene (ABS) or polyoxymethylene (PC) filaments. Both particles and VOCs were collected using an impinger sampler. Samples were characterized for their physicochemical properties, cellular cytotoxicity, oxidative stress response, apoptotic effects, and cytokine production. Results showed that printers with PC filaments generated two-fold more particles/ml than ABS. Mean sizes of PC and ABS-emitted particles in cell culture media were 201 ± 8 nm and 198 ± 10 nm, respectively. Bisphenol A and styrene were the predominant VOCs collected in the media for the PC and ABS emissions, respectively. At 24 h post exposure, both PC and ABS emissions elicited significantly increased cytotoxicity, with PC being more toxic than ABS. Moreover, PC induced higher production of reactive oxygen species, and decreased in total antioxidant capacity and glutathione peroxidase activity than ABS. Furthermore, both PC and ABS emissions induced apoptosis in SAEC with the PC emissions induced four-fold more apoptotic cells than the ABS emission. Cytokine and chemokine profiling showed that PC emissions induced higher production of seven proinflammatory cytokines and chemokines than ABS. Taken together, the results indicate that the emissions generated by PC and ABS filaments induce toxicity in SAEC, and the exposure to the PC emission induces more toxicity than that of the ABS emission.

3070 Capture Compound Mass Spectrometry: Elucidating Off-Target Binding to Deconvolute Drug Toxicity


Understanding both the on- and off-target protein binding interactions of small molecules is an essential part of the drug discovery process. A large proportion of toxicity findings in non-clinical or clinical studies are precipitated by the parent drug or a metabolite binding an off-target protein target, such as an enzyme or receptor, modifying its function resulting in cellular dysfunction and toxicity. Capture Compound Mass Spectrometry (CCMS) is an unbiased, proteome-wide approach for the identification of specific-binding protein targets for small molecules and peptides. The technology combines medicinal chemistry and in vitro pharmacology, coupled to high resolution proteomics mass spectrometry to isolate and identify target proteins that are responsible for an observed biological response. Thus, through in vitro investigation in target tissues of interest, the candidate proteins and pathways causing in vivo toxicity can be elucidated. An overview of CCMS technology and its application in identification of off-target compound activity is presented. The CCMS technology has been used to determine on- and off-target interactions at the 4-aminopyridine (APY) and tolcapone (COMT) inhibitor tolcapone in a human liver cancer cell line (HeP2). A comprehensive interaction profile was generated, revealing both on- and off-target binding proteins. Differential profiles of tolcapone which causes liver toxicity and entacapone that does not were elucidated and highlighted 3-hydroxyisobutyryl-CoA hydrolase (HIBCH) as a candidate target mediating toxicity. Medicinal chemistry was then initiated focusing on molecules without HIBCH activity resulting in ‘tolcapone-like’ molecules with reduced toxicity profiles that could lead the way to the development of improved COMT inhibitors.

3071 A Practical High-Throughput Co-Culture Plate to Screen Paracrine and Endocrine Interactions

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Traditional HTS in microtiter plates is limited to one cell type, excluding important paracrine and endocrine cellcell interactions, while Transwell® inserts are too cumbersome with 30 wells and 15° well robotic HTS. We have developed a microtiter plate based co-culture system dubbed MICRO-MT as a solution to fulfill the HTS co-culture and multi-culture needs of the drug discovery and toxicity testing market. In the MICRO-MT, cells are monocultured in individual wells of a microtiter plate with standardized dimensions as usual, but by simply increasing fluid volume, the media from monocultures in adjacent wells are bridged through the integrated microchannels allowing co-culture through diffusion of metabolites between wells. Because the MICRO-MT operates by simple diffusion and in a traditional microtiter plate footprint, it doesn’t require additional equipment beyond that already in use and is compatible with existing HTS infrastructure. To illustrate the utility of this approach, we developed a conferred steroid metabolism assay using an estrogen receptor driven luminescent reporter cell line, MVLNs alone or in co-culture with HepG2 cells. HepG2 cells contain high levels of aromatase activity which converts testosterone to estrogen, while MVLN cells do not express aromatase. MVLN cells in monoculture treated with 17β-estradiol ranging 100pM to 10nM showed high luminescence activity, whereas testosterone treatment showed no luminescence activity as predicted. In contrast, co-culture of MVLN and HepG2 cells in the MICRO-MT showed dose-responsive increases in MVLN luminescence activity in response to testosterone treatment (EC50 = 4.1nM) indicating HepG2 cells converted testosterone to estrogen which then diffused in the reporter well containing MVLN cells activating the luminescent reporter. We have also found that this platform recapitulates a paracrine Sonic Hedgehog (SHH) signaling response, whereby SHH ligand produced from GMSM-K SHH transfected epithelium generates robust SHH pathway activation in cultured SHH reporter cells that combine for antagonists vismodegib and cyclopamine, but also secretory antagonists Ruskii-43 and U18666A. These studies show that the MICRO-MT plate is a viable solution to support paracrine and endocrine interactions in a technically simple format that is amenable to HTS.

3072 Evaluation of Transdermal Drug Delivery and Toxicity in a Microphysiological Body-on-a-Chip System


Body-on-a-chip (BoaC) in vitro systems are a promising technology for increasing the predictive power of drug efficacy and toxicity in humans compared to traditional animal models. These interconnected multi-organ systems can be improved by expanding drug delivery methodologies to include oral and transdermal applications. Towards that goal, we have developed a heart-lyer-"skin" BoaC system to assess the toxicity of topically administered drugs dosed acutely and chronically. To validate the topical delivery system, the moderate permeation drug diclofenac (1.5 and 3% solutions), and the low permeation compounds ketocanaol (0.11%), hydrocortisone (1%), and aspirin (1.5%) were applied to a synthetic skin surrogate (Strat-M). Understanding both the on- and off-target protein binding interactions of small molecules is an essential part of the drug discovery process. A large proportion of toxicity findings in non-clinical or clinical studies are precipitated by the parent drug or a metabolite binding an off-target protein target, such as an enzyme or receptor, modifying its function resulting in cellular dysfunction and toxicity. Capture Compound Mass Spectrometry (CCMS) is an unbiased, proteome-wide approach for the identification of specific-binding protein targets for small molecules and peptides. The technology combines medicinal chemistry and in vitro pharmacology, coupled to high resolution...
3075 Combining Trans-Epithelial Electrical Resistance (TEER) Measurements with a Microfluidic Gut-on-a-Chip System for Real-Time Assessment of Drug-Induced Barrier Disruption


Intestinal barrier disruption resulting from drug-induced toxicity can lead to life-threatening conditions and the cancellation of important therapeutic programs. Current in vitro 2D intestinal models lack the features of the in vivo settings like tubular structure or perfusion, and fail in clinical translation. Hence there is an urgent need for reliable epithelial barrier models and translatable, real-time readouts from these predictive in vitro systems. Here we present extracellular matrix-supported intestinal tubules in a perfused microfluidic platform, that are fully-compatible with trans-epithelial electrical resistance (TEER) measurements over the full duration of the experiment. These intestinal tubules exhibit tissue (polarization) markers ErbB1, ErBb2, Ezrin, Glut-2, MRFP and ZO-1, and crypt-like morphology. Further, these tissues exhibit relevant TEER values (~200 Ω/cm²) prior to compound exposure, while reaching maturation significantly faster than Transwell-based models (4 days vs. 21 days). Drug-induced toxicity was assessed by apical exposure of the intestinal barriers to model compounds, as well as to cytokines to mimic inflammatory, immune-mediated gut toxicity. A fully-automated TEER measurement platform was applied during the exposures, tracking barrier disruption in up to 96 gut tubules cultured in parallel and facilitating characterization of a dose- and exposure time-dependent response for predicting gut toxicity. The described techniques have been designed to be readily implemented in routine laboratories and high-throughput facilities, allowing for enhanced prediction of the clinical response to pharmacological stimuli in real-time.

3073 Comparing Tanimoto Coefficient Values Calculated by Toxmatch and OECD QSAR Toolbox

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The Tanimoto Coefficient is a measure of similarity between a query chemical and other chemical structures of interest. It is valuable tool to limit the number of analogue chemicals when performing read-across. As part of a Tanimoto Coefficient calculation, a chemical is broken down to structural or molecular fragments, which are based on pharmacologically known or predefined fingerprints. A “fingerprint” of a chemical is an ordered list of binary (1/0) bits, which records the presence (“1”) or absence (“0”) of the predefined fragment in the molecule. Fingerprints of two phthalate esters are compared for similarity using the Tanimoto Coefficient (T). The Tanimoto Coefficient varies from zero to one in value, with a coefficient closer to one indicating that the two chemicals share identical hits or fragments. Cutoffs of 0.6 have been proposed in the evaluation of structurally similar chemicals and the risk of teratogenicity, an endpoint that has poorly defined structural alerts. The Tanimoto Coefficient can be calculated using the Toxmatch program, an open-source software application that encodes several chemical similarity indices to facilitate the grouping of chemicals into categories and rank order for chemical similarity calculations. Similarly, OECD QSAR Toolbox, an open-source software that works by grouping chemicals into categories and then allowing the user to progress through the workflow by identifying relevant structural characteristics and/or potential mechanisms or modes of action of the target chemical, can be used to calculate the Tanimoto Coefficient. In a case study, Tanimoto Coefficients were calculated using both the Toxmatch and OECD QSAR Toolbox programs to compare the similarity between mono-(2-hydroxyethyl) terephthalate (CASRN 1137-99-1) and dimethyl terephthalate (CASRN 120-61-6). Toxmatch returned a value of 0.763, while OECD QSAR Toolbox returned a value of 0.45. As demonstrated through this comparison, calculation of Tanimoto Coefficients between molecules can vary, depending on the molecular features and atom specifications used to create the fingerprint. Toxmatch bases its fingerprinting on atom environments, while OECD QSAR Toolbox uses the default characteristics of atom centered fragments (molecular feature) and atom type, count H attached and hybridization (atom characteristics). This case example demonstrates differences between two software programs and underscores the importance of incorporating multiple software tools when employing read-across methods to identify and select chemical surrogates.
3077 Application of the Public Health Exposome Framework to Estimate Endo-Phenotypes of Resilience in a Model Ohio African-American Woman’s Cohort

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We report integration of the US EPA EJSSCREEN database with our Public Health Exposome dataset to interrogate 9,232 census blocks to model the complexity of relationships among environmental, and socio-demographic variables towards estimating adverse pregnancy outcomes (low birth weight, LBW and pre-term birth, PTB) in all Ohio counties. Using a hill-climbing algorithm in R software, we derived a Bayesian network that mapped all controlled associations among all variables available by applying a mapping algorithm. The results revealed 17-environmental, and socio-demographic variables that were represented by nodes containing 69-links accounting for a network with 32.85% density and average degree of 9.2 showing the most connected nodes in the center of the model. The model predicts that the socioeconomic variables low income, minority, and under age five populations are correlated and associated with environmental variables; particulate matter (PM10) level in air, proximity to risk management facilities, and proximity to direct discharges in water and are linked to PTB and LBW in the 88-Ohio counties. The methodology used to derive significant associations of chemical and non-chemical stressors linked to PTB and LBW from indices of geo-coded environmental neighborhood deprivation serves as a proxy for design of an African American women’s cohort to be recruited in Ohio from federally qualified community health centers within the 9,232 census blocks. The results have implications for the development of severity scores for endo-phenotypes of resilience based on associations and linkages for different chemical and non-chemical stressors that have been shown to moderate cardio-metabolic disease within a population health context.

3078 Dietary Influence on the Metabolism and Biological Effects of Arsenic Exposure: A Double-Blind, Placebo-Controlled Folate Supplementation Trial in Inner Mongolia, China

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Chronic arsenic exposure via drinking water is a worldwide public health concern and is associated with increased morbidity and mortality from both non-cancerous and cancerous effects. Increasing evidence suggest that the intake of dietary nutrients has an impact on arsenic metabolism and DNA methylation, which could lead to differential susceptibility of individuals to the toxic and carcinogenic activity of arsenic. Folate, one of dietary methylation, which could lead to differential susceptibility of individuals to the toxic and carcinogenic activity of arsenic before and during the study periods. These participants were enrolled in a double-blind, placebo-controlled folate acid supplementation trial in Hetao Plain of Inner Mongolia, China. We got consent and enrolled three hundred adult participants who were exposed to arsenic before and during the study periods. These participants were enrolled and randomly divided into three groups and given folate acid at a dose of 400 or 800 µg/d, or placebo for 8 weeks. After the trial, in contrast to placebo group, subjects’ serum folate level and plasma homocysteine concentration significantly increased and decreased, respectively, in both folate acid supplementation groups; these individuals also showed a stronger arsenic metabolism capability, featured by the lower percentage of urinary iAs (inorganic arsenic) and MMA (monomethylarsonic acid) and higher percentage of urinary DMA (dimethylarsonic acid). Furthermore, a dose-dependent effect of folate was observed for folate between supplementation dosage and arsenic metabolism efficiency. In addition, folate supplementation was associated with an increased global DNA methylation level of peripheral blood. In conclusion, folate supplementation has a positive effect in facilitating arsenic biometabolism and maintaining global genome DNA methylation levels. The work was supported by NIEHS grant R21ES022329 and R01ES022629 to X.R.
AM blood concentrations from PM blood THC concentrations. Our weight of evidence analysis indicates current scientific data do not support prediction of AM blood concentrations from PM concentrations.

**3081** A Case-Control Study of Newborn Hearing Screen Outcomes and Neonatal Dried Blood Spot Metals

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Toxicant metal exposures, such as lead (Pb) and mercury (Hg), have been associated with hearing loss in adults and youth. However, little is known about the role of prenatal exposures to Pb and Hg in hearing health during human fetal development. This case-control study explored the relationship between newborn newborn hearing screen pass/fail outcomes and newborn whole blood levels of two non-essential metals (Pb and Hg) along with six essential elements (calcium, copper, iron, potassium, selenium, and zinc). The Michigan Early Hearing Detection and Intervention Program provided data on 338 infants with an abnormal hearing screening result and 338 infants with a normal hearing screen result. All infants were born between 2003 and 2015. Cases and controls were matched by birth year, sex, and race by the Michigan Neonatal Biobank, which also provided the dried blood spots for each infant. Toxic and essential elemental levels in the newborn dried blood spots were determined by direct mercury analysis (DMA) for mercury and total reflection-X-ray fluorescence for metals. Blood metals were corrected for body weight and height. A novel statistical approach, principal component analysis (PCA), was used to perform a data-driven assessment of the relationship between metals and hearing screen outcomes. The findings suggest that neonatal blood calcium levels may be important determinants of hearing health, though further studies are warranted to understand the physiological mechanisms. These findings need to be interpreted with caution because this matched case-control study was unable to control for genetic causes of hearing loss, cytomegalovirus infection, or cranio-facial abnormalities, all which can cause hearing impairment. The present study contributes to the current knowledge that dried blood spots, while a novel metric, have merit for further use in determination of nutrient levels and environmental exposures.

**3082** Amniotic Fluid Exposomics Identifies Novel Prenatal Exposures and Metabolic Signatures in Relation to Preterm Birth: A Prospective Case-Control Study

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Preterm birth (PTB, gestation age < 37 weeks) is a leading cause of neonatal mortality and long-term perinatal morbidity and is a top public health priority in the U.S. and worldwide. Despite advances in risk factor characterization, biomarkers for early diagnosis and effective prevention are lacking, so are the potential role of environmental exposures. Surprisingly, only a few classes of environmental agents have been studied to date, all in a targeted manner, as potential etiologic contributors to PTB, leaving the effects of ubiquitous yet complex prenatal exposures largely unexplored. Here, we conduct an untargeted exposomic investigation on PTB. Prospective amniotic fluid samples (n=21 cases; n=21 controls) were collected, extracted, and profiled by high-resolution accurate-mass (HRAM) mass spectrometry platform coupled to a state-of-the-art structural identification workflow. Differential case-control abundances were defined by Welch t-test (p < 0.05) and the association of abundance with gestation age was assessed via Pearson correlation. The study yielded a panel of compounds of which the abundance in amniotic fluid was negatively associated with gestational length. Of note, xenobiotic prenatals exposures embrace a large diversity by source, structure and health effects, ranging from microbial metabolites (e.g. 1,8-diazacyclotetradecane-2,9-dione), plant-derived alkaloids (e.g. N-methyllycine), to synthetic organic chemicals (e.g. diclofenac). Likewise, novel metabolic signatures were observed as well. Notable examples include cortisol (altered maternal stress levels), sphenamine (perturbed uterine contraction), hypoxanthine and uric acid (indicating hypoxia), and an array of lysophospholipids (lysOPC) (associated with PTB and fetal health). This exposomics study comprehensively characterizes prenatal exposures alongside metabolic features in relation to PTB, which may be valuable to inform strategies to develop new biomarkers and decrease prenatal exposures.

**3083** Diabetes and Persistent Organic Pollutants in the Anniston Community Health Survey Follow-Up

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In 2014, we conducted a follow-up study (ACHS II) of the Anniston Community Health Survey (ACHS in 2005-7) where residents’ polychlorinated biphenyls (PCBs) concentrations were about 3 times higher than in the general U.S. population. Results from ACHS showed significant associations between non-dioxin-like PCBs and diabetes. ACHS II included measurements of dioxins in addition to PCBs and pesticides. Toxicological studies have explored inflammatory response and insulin signaling disruption, glucose homeostasis and pancreas cell function, as well as the disruption of adipogenesis by which exposure to POPs may lead to the development of diabetes. Serum samples and covariate information were available for 338 participants. Diabetes status was defined as being on glycemic medication or having a glucose concentration greater than 125 mg/ml; 135 (39.9%) of ACHS-II participants were diabetic in 2014. The polychlorinated dibeno-p-dioxins (PCDD), dibenzofurans (PCDF), and non-ortho PCBs were measured using high-resolution gas chromatography/high-resolution mass spectrometry and expressed as total dioxin toxic equivalents (TEQs, pg/g lipid). Pesticides measure included pp'-DDE, hexachlorobenzene, β-HCH, oxychlordane, trans-nonachlor, and mirex. Odds ratios (OR) and 95% confidence intervals (CI) were estimated using unconditional logistic regression models. No consistent, significant relationships were observed for hearing screen failures for levels of Pb (OR = 1.04, 95% CI: 0.86-1.26) or Hg (OR = 1.09, 95% CI: 0.91-1.31). In infants with higher levels of blood spot calcium did show significantly decreased odds of hearing screen failure (OR = 0.49, 95% CI: 0.34-0.70). Other essential elements were not significantly associated with odds of hearing screen failure. These findings suggest that neonatal blood calcium levels may be important determinants of hearing health, though further studies are warranted to understand the physiological mechanisms. These findings need to be interpreted with caution because this matched case-control study was unable to control for genetic causes of hearing loss, cytomegalovirus infection, or cranio-facial abnormalities, all which can cause hearing impairment. The present study contributes to the current knowledge that dried blood spots, while a novel metric, have merit for further use in determination of nutrient levels and environmental exposures.

**3084** Urinary Metals and Pre-eclampsia in the LIFECODES Birth Cohort: A Single-Contaminant and Mixture-Based Approach

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Pre-eclampsia (PE) is a major contributor to maternal mortality. Exposures to toxic metals and deficiencies in essential metals have previously been linked to increased risk of PE. However, the effect of exposure to combinations of toxic metals remains unknown. In this study we evaluated the relationship of individual trace metals, alone and in mixtures, and PE was investigated using 383 subjects from the LIFECODES prospective birth cohort, which included 28 women who experienced PE. Urine samples collected during pregnancy (median, 26 weeks gestation) were analyzed for 17 trace metals. We created Cox proportional hazard models to calculate hazard ratios (HR) of PE in association with the concentration of individual urinary trace metals. As a mixtures-based approach, we used principal components analysis (PCA) to identify components of trace urinary metals and their associations with PE. In addition, interactions between toxic and essential metals were investigated. In single-contaminant models, the HR (95% CI) associated with urinary chromium was 4.02 (1.10, 14.8). Although the overall relationship between urinary cadmium and PE was null, urinary cadmium was associated with an elevated risk of PE among women with low urinary selenium (HR: 2.52, 95% CI: 1.00, 6.32). In PCA, metals loaded onto 3 principal components, characterized by loading from Essential Metals (PC1: copper, selenium, and zinc), Toxic Metals (PC2: lead, manganese, and cadmium), and Seafood-Associated Metals (PC3: arsenic, mercury, and tin). Individually, these components were not associated with the risk of PE. However, among individuals with low Essential Metals (PC1), Toxic Metals (PC2) were associated with an increased risk of PE (HR: 2.22, 95% CI: 1.06, 4.64). Therefore, the relationship between prenatal toxic metal exposure and PE incidence may be modified by levels of essential metals.
Human Developmental Neurotoxicity of Chlorpyrifos: Quantitative Evaluation of Exposure


As part of a comprehensive risk assessment of chlorpyrifos, the California Department of Pesticide Regulation (DPR) examined the evidence for developmental neurotoxicity of this organophosphate insecticide in human epidemiological studies. Several ongoing prospective studies as well as some more recent observational studies have investigated the association between perinatal chlorpyrifos exposure and the potential for altered human growth and behavior later in life. The exposure-effect associations were evaluated using the following: 1) the choice of biomarker of exposure; 2) the timing of biological sampling in relation to the putative exposure and/or developmental effects; 3) variations in quantitative measurements within and across individuals; and, 4) the analytical methodologies utilized. The epidemiological studies were conducted in the United States as well as in the Philippines, China, and Mexico and measured either the parent compound in blood, hair, and meconium, or one or more metabolites such as 3,5,6-trichloro-2-pyridinol (TCPy) in blood and urine. There was considerable variation in the choice of biomarkers as well as the analytical methodologies and sensitivities of measurements across studies. Several of the most recent studies reported non-detection of chlorpyrifos in maternal and fetal samples. In some of the older studies that detected parent compound in biological samples, the standard curves terminated above the limit of detection/quantitation (LOD/LOQ) and occasionally the median biomarker values were below the LOD or LOQ. Findings from environmental epidemiology studies are becoming more frequently considered in human health risk assessment. However, doing so depends on the quality of the exposure analysis. Because of the limitations in the available data, DPR was unable to reliably discern an exposure-effect relationship or derive a regulatory target for chlorpyrifos based on human epidemiological data at this time.

Military Occupation as a Surrogate Measure of Potential Exposure to Fuels and Associated Long-Term Health Effects

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The US Department of Veterans Affairs (VA) has received several inquiries regarding the potential health effects of exposure to fuels and their components during military service. Studies conducted by the US Department of Defense (DoD) have characterized the acute effects of fuels on service members; however, there is a need to better understand the potential long-term health consequences of chronic exposure. The purpose of this study was to determine the ability of investigating the long-term health effects of occupational fuel exposure in Veterans who served in the Air Force (AF). Occupation was used to define levels of exposure, and associations between occupational exposure to fuels and chronic health outcomes were assessed, including, but not limited to, potential effects on the auditory and visual function, other effects on the nervous system, hematological effects, respiratory effects, effects on immune function, and cancers. The health data from several VA and DoD databases were linked to ascertain these outcomes. Occupation proved to be an adequate surrogate for fuel exposure, as long-term health effects, such as chronic deficits in hearing, were identified in Veterans who had a high likelihood of exposure based on their duties. This feasibility study will serve as a "proof-of-concept" for a more comprehensive, DoD-VA, multidisciplinary investigation into the consequences of occupational exposure to fuels during military service.

Alterations in Profiles of Metabolome and Gut-Microbiome Due to Exposure of PCBs and p,p'-DDE in C-MACH Cohort

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Exposure to environmental chemical, like persistent organic pollutants (POPs) has been associated with adverse human reproductive and fetal developmental outcomes. However, the biological mechanisms of adverse effects of these compounds in humans are not currently well established. In this study, we aimed to identify the altered biological pathways by exposure of PCBs and p,p'-DDE, in pregnant women using a metabolome profile in maternal serum and feces, and gut microbiota profiles. Pair of human serum and feces sample was corrected from 58 participants around 32 weeks of gestational age in “Chiba Study of Mother and Child Health (C-MACH)" consisted of three hospital-based cohorts. Data from participants were available for 51of the 58 maternal participants. Serum concentrations of PCBs, serum and feces metabolome, and gut microbiota were analyzed by GC-MS, LC-MS/MS, GC-MS/MS, and next-generation sequencing system, respectively. Dietary habits were corrected using a brief diet history questionnaire (BDHQ). We used the DIABLO (Data Integration Analysis for Biomarker discovery using a latent component method (OMICS studies)) models, and patients with the highest exposure levels of contaminants (first [Q1], second-third [Q2-3], and fourth quartile [Q4]) for pregnant women. The levels of PCBs (Mean: 450 pg g⁻¹ wet wt, Q1 < 280 pg g⁻¹ wet wt, Q2-Q3: 280-560 pg g⁻¹ wet wt, Q4: > 560 pg g⁻¹ wet wt) and p,p'-DDE (Mean: 440 pg g⁻¹ wet wt, Q1 < 290 pg g⁻¹ wet wt, Q2-Q3: 290-630 pg g⁻¹ wet wt, Q4: > 630 pg g⁻¹ wet wt) were lower than previous reports from the USA, EU, and Japan. In the classification DIABLO model for PCBs, p,p'-DDE for classification had > 0.72 AUC values. Interestingly, PCB and p,p'-DDE levels in maternal serum were strongly correlated; however, networks of related compounds were not well overlapped. Focus on candidate biomarkers and metabolite included in composited models for PCBs were related to microbiome, serum and feces metabolome profiles. On the other hand, p,p'-DDE was related to serum metabolome profiles.

Impact of the Opioid Epidemic and Fentanyl Related Overdose Cases in a Florida West Coast Medical Examiner District


Opioids are characterized by a high level of misuse and abuse with a high dependency and risk of overdose. Opioids can be divided into two classes, natural and synthetic, both classes acting on the mu opioid receptor. Fentanyl is a potent synthetic opioid that is roughly 50 to 100 times more potent than morphine. The United States is currently experiencing an opioid epidemic that does not show any signs of waning. The opioid epidemic’s impact has been so profound that evidence suggests it has caused a decrease in life expectancy for men. The current study evaluates trends in opioid use in overdose cases in the Florida District 6 Medical Examiner’s office and the role of fentanyl in overdose related mortality. Oxycodeone was present in 248 cases in 2011 in District 6. That number dropped to 108 cases in 2016. Cases with hydrocodone present demonstrated a similar profile, decreasing from 88 in 2011 to 28 in 2016. At the same time, in District 6 there was a 51.7% increase in deaths involving fentanyl or a fentanyl analog from the year 2011 to 2016. In 2011, 71% of the cases were men; 98% of those men were white. In 2011, the average age of fentanyl involved death was 44; in 2016, the average age was 38. The population most affected by the surge in fentanyl overdoses were men between the ages of 25-35 years old. The spike in fentanyl and fentanyl analog related deaths have caused difficulties from an analytical toxicology perspective. Fentanyl is an extremely potent drug, and thus very small amounts are required to cause accidental overdose compared to natural opioids such as morphine. The results suggest that fentanyl and fentanyl analogs are an emerging priority in opioid epidemic research.

Evaluating the Impact on IQ of Short-Term Increases in Blood Lead Levels


Significant research has been conducted on the relationship between blood lead levels in young children and IQ (e.g. Lanphear et al., 2005). Governmental agencies have relied on determinations of the blood lead – IQ association to set environmental exposure levels for lead. However, little has been done to protect young children from elevated blood lead levels. However, an unanswered question is the degree to which long-term average vs. short-term elevations in blood lead levels are more strongly associated with the observed IQ deficits. Available datasets are for children with long-term exposures to lead. However, children in these datasets who have peak blood lead levels substantially higher than their life-time average, consistent with short-term exposure, can be identified and analyzed separately. This analysis was conducted with the Lanphear et al. (2005) pooled blood lead - IQ dataset, with corrections described by Crump et al. (2013). The data were divided by establishing a threshold between two subsets defined by peak blood lead = 10.9 + 1.6 x concurrent blood lead, where approximately 10% of the data fall above the boundary. Analyses of the full datasets using the Lanphear log-linear model reproduced the coefficients and R²’s reported in Table 2 of Crump et al. The same model was run for the data subsets, regressing both peak blood lead
and concurrent blood lead against IQ. The chronic exposure data subset analyses have similar regression coefficients and R² values as reported in Crump et al. for the full dataset. Both regressions for this data subset are statistically significant. In contrast, the regressions of peak blood lead and concurrent blood lead vs. IQ for the acute exposure data subset are not statistically significant, and have positive rather than negative regression coefficients. This indicates that peak blood lead levels that are short-term are not correlated with IQ. However, concurrent blood lead levels for the acute exposure data subset are also not correlated with IQ. Several alternative analyses were also conducted, including with different blood lead metrics, different boundaries between data subsets, and with removing outliers. For all alternatives, regression results were similar. Possible explanations for the lack of correlation between blood lead and IQ for the acute exposure data subset will be presented. In conclusion, the results suggest that long-term chronic exposures to lead are more closely associated with IQ impacts than are short-term blood lead elevations.

3090 Environmental and Genetic Drivers of Telomere Length Variation in Ethnically Diverse Africans

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Telomeres are repetitive non-coding sequences at the ends of chromosomes that protect DNA from damage. Each time a cell divides, telomeres progressively shorten, until a critical length is reached and cell division stops. Thus, telomere length is closely associated with aging and lifespan. In addition, longer telomeres are associated with increased risk of many cancers, while shorter telomeres are associated with elevated cardiovascular disease risk. Finally, telomere length varies with environmental factors, including chronic stress and infection status. However, relatively little is known about the genetic architecture underlying telomere length, or the environmental factors that influence telomere loss. Here, we investigate the relationship between telomere length, genetics, and environmental factors in a set of ethnically diverse African people (n=1820) originating from populations in Botswana, Tanzania, Ethiopia, and Cameroon. We find significant variation in telomere length among populations after adjusting for age and sex, with the San hunter-gatherers from Botswana having the longest telomeres, and individuals from Cameroon having the shortest telomeres. Telomere length also varies with environmental factors, including chronic stress, in a way that is consistent across populations. Finally, when we analyze the relationship between telomere length and malaria endemicity, precipitation, and temperature variables. Finally, after accounting for genome-wide ancestry and relatedness among individuals, we find that a large proportion of inter-individual variation in telomere length (>40%) is explained by genetic factors. Ongoing work examines the relationship between telomere length and other phenotypes, including cardiovascular traits, as well as whether patterns of selection at genetic loci underlying telomere length vary with environmental factors across Africa. This research will help elucidate the evolutionary forces driving telomere length variation in humans, which will provide insight into the basis of telomere-related disease risk.

3091 Examination of the US FDA Adverse Event Reporting System to Assess the Halo Effect and Potential Reporting Bias

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The US Food and Drug Administration (US FDA) maintains an adverse event reporting system with data collected from users of various food, beverage, and cosmetic products. Recently, there have been concerns regarding alleged adverse health effects among users of WEN by Chaz Dean (WCD) hair cleansing conditioners, including hair loss, hair breakage, and skin sensitization and irritation. The objective of this analysis was to analyze the temporal trends in reported adverse event data specific to WCD cleansing conditioners before and after media coverage of alleged health effects in 2014. Publicly available data were extracted from the US FDA Center for Food Safety and Applied Nutrition (CFSAN) Adverse Event Reporting System (CAERS) from 2004 to 2018. Data were restricted to WCD-specific products, and were further limited to potential cleansing conditioner products. A total of 1,903 adverse events were reported among the products that fit the study inclusion criteria. The most prevalent reported adverse events were alopecia, pruritus, trichorrhexis, and rashes. Out of the total number of WCD-specific reported adverse events, 8.6% were reported to occur in or after 2014. Additionally, based on the available company sales records, a subset of adverse event data (2005 to 2015) was analyzed using negative binomial regression. The rate of adverse event reporting specific to WCD cleansing conditioners after 2014 was statistically significantly higher in comparison to the rate of adverse event reporting before 2014, adjusting for the number of hair cleansing conditioner units sold per year and the number of reported non-WCD-specific adverse events (to account for temporal trends in the adverse event reporting system). These findings suggest the potential for a halo effect, where negative news media may alter reporting behaviors due to societal shifts in product-specific risk perception.

3092 Increased Opioid-Related Overdose Deaths Are Associated with Increased Abuse of Illicit Fentanyl in the United States

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Opioid-like substances are agonists binding the opioid receptors of neurons in the human central nervous system and serve as painkillers in clinical medicine. However, there has been a significant increase in the illegitimate abuse of fentanyl-like substances in recent years, which poses serious health risks to the general public. To better understand the use of illicit fentanyl-like substances, and its impact on public health including its correlation with opioid overdose deaths in the US, a systematic review from epidemiology databases was conducted and analyzed. The cohort datasets were collected from two major public health surveillance systems, including (1) The US’ Center for Disease Control and Prevention (CDC)’s Morbidity and Mortality Weekly Report (MMWR) during the years from 2010 to 2017 and (2) the data published by the National Forensic Laboratory Information System (NFLIS), which systematically collects results from laboratories nationwide that monitor the illicit use and exhibit of psychoactive substances. The CDC reported that 21,088 opioid overdose deaths in 2010 increased to 49,068 deaths in 2017, with a 13.08% average annual increase. The NFLIS reported 579 cases of illicit synthetic fentanyl use in 2010, increasing over 123-fold to 71,431 reported cases in 2017. The study results from both the CDC’s MMWR and NFLIS demonstrated a substantial increase in the occurrence of opioid-related overdose deaths and the use of illicit synthetic fentanyl in the US. In addition, there was a drastic increase in several synthetic fentanyl-like substances (i.e. acetyl fentanyl, acryl fentanyl, butyl fentanyl, furanyl fentanyl, etc.) with non-medical approval in the last four years. A correlation study shows that the increase in illegitimate use and exhibit is positively correlated with the increase in overdose death rates in the US. The analytic result is statistically significant. The results offer substantial evidence that the increased use of illicit fentanyl may positively contribute to the increased death rate due to opioid overdose. Based on this preliminary study, a further regulatory assessment or legal enactment may help reduce the risk to public health.

3093 The Risk of Lung Cancer Due to Occupational Exposure to Talc: A Meta-analysis of Miners and Millers

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It has been suggested that occupational exposure to talc may be associated with lung cancer. However, none of the previous meta-analyses assessed the influence of smoking on this potential association. To address these concerns, an updated systematic review and meta-analysis of six miner and miller cohorts exposed to talc and their risk of lung cancer was conducted. The goal of this study was to evaluate whether a causal association exists between lung cancer and occupational exposures to talc from mining and milling processes. Specifically, four cohort studies (five occupational groups) were evaluated exposures to cosmetic talc and one cohort study evaluated industrial talc exposures. Without smoking adjustment, meta-analyses showed no increased risk of lung cancer for miners or millers separately. However, when the risk for miners and millers were pooled a statistically significant increased risk of lung cancer was observed (meta-RR = 1.438, 95% CI: 1.063, 1.943). Subsequently, indirect adjustment techniques were used to account for smoking behaviors among studies for which the initial lung cancer risks were not adjusted for smoking behavior. Adjustment for smoking lowered individual risk estimates for miners and millers and led to a reduction in lung cancer risk for the pooled miner and miller estimate such that the association was no longer statistically significant (meta-RR = 1.186, 95% CI: 0.844, 1.667). When assessing exposures to cosmetic talc alone, none of the meta-RRs showed a statistically significant increased risk of lung cancer, regardless of occupational designation. The results of this study demonstrate the importance of accounting for smoking behaviors when evaluating causal relationships between occupational expo-
sures and the development of lung cancer. In conclusion, this systematic re-
view and meta-analysis found no evidence to support a causal association be-
tween exposure to talc while employed as a miner or Miller and lung cancer.

3094 Five-Year Lung Cancer Mortality Risk Analysis and Topography in Xuan Wei: A Spatio-
temporal Correlation Analysis
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China.

In Xuan Wei, China, the lung cancer mortality rate is rising significantly higher 
than the whole nation significantly. However, the improved diagnosis just 
can explain partially, other local risk factors might exist. This study inves-
tigates the spatial and temporal trends of lung cancer in Xuan Wei from 2011 
to 2015. Spatial autocorrelation was explored. Four sets of spatial analysis 
and spatial statistics were applied: 1) hotspot analysis and 3D-geographical 
visualization, 2) spatially weighted sum, 3) spatial interrelation between coal 
mines and lung cancer mortality; and, 4) a geographically weighted regres-
sion model. Females exhibited higher lung cancer mortality than males, with 
an increasing trend observed for both genders over time. The incidence rate 
in Laibin Town was the highest in Xuan Wei for each year. Higher mortality 
was found in the towns surrounded by the coal industries with smoky coal 
mines. The hotspot analysis showed the lung cancer mortality has been in-
creasingly concentrated at Laibin, Shuanglong and Longchang over the 5 
years. The 2D and 3D mapped health risks showed the geographical pattern 
of potential lung cancer health risks from the coal mine. The results of correla-
tion analysis illustrate that there was no significant correlation between lung 
cancer mortality as a whole and coal mine distribution for the five years span. 
However, the geographical weighted regression models revealed the stron-
ger correlation in the specific areas where smoky coal mines are concen-
trated. Lung cancer mortality has increased continuously since the third mor-
tality survey in Xuan Wei, especially for local females and the residents over 
35 year olds. Geographically, the locations for different kinds of mines have 
interrelation with the lung cancer mortality there. The specific areas within 
Xuan Wei demonstrated high correlations between lung cancer mortality and 
coal mines. Strategy for intervention and additional studies are warranted to 
systematically examine the local environmental health risks related to coal 
combustion indoor air pollution and eventually to conduct the early screen-
ing of lung cancer for local people who use the smoky coal in the high-risk 
spatial areas.

3095 Optimization of an In Vitro CYP450 Induction 
Assay Using Cryopreserved Cell Lines
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Cytochrome P450 (CYP450) enzymes are responsible for the majority of Phase I metabolism. Induction occurs when a drug increases the rate of CYP450 enzyme production. As a result, the rates of metabolism of subsequent drugs increase, posing concerns for dosing and safety. To further expand the USAMRICD Absorption, Distribution, Metabolism, Excretion, and Toxicity 
Center of Excellence (ADMET CoE) profiling capabilities, a CYP450 induction assay is being developed using alternative cell lines to primary human he-
patocytes to measure changes in gene expression of CYP1A2 and CYP3A4 isoenzymes. Primary human hepatocytes are the gold standard for evaluating P450 induction in vitro; however, they have inherent limitations such as high 
costs, a fastidious nature, and significant lot-to-lot variability. Induction as-
sessments using cryopreserved hepatocytes and two immortalized cell lines, 
HepaRG and HepatoCells, are being conducted to determine a more robust, 
cost-effective alternative model. Each cell line was exposed to the standard 
inducers rifampicin and omeprazole to assess CYP3A4 and CYP1A2 activ-
ity, respectively. Following a 72-hour incubation period, RNA from the cell 
cultures was extracted and purified. Fold changes of gene expression were 
measured using real-time quantitative PCR (RT-qPCR). Hepatocytes displayed an average fold change of 92.7 for CYP3A4 and 49.6 for CYP1A2, whereas 
HepaRG displayed changes of 28.6 and 179.5, respectively. Average values for CYP3A4 and CYP1A2 for HepatoCells were 58.7 and 43.9. Based on the 
data, HepatoCells most closely mimicked cryopreserved hepatocytes in com-
parison to HepaRG cells. Additional data on assay parameters and technique 
optimization will be presented. Opinion disclaimer: The views expressed in this 
abstract are those of the authors and do not reflect the official policy of the US 
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3096 Comparison of Cytochrome P450-Related 
NADPH Oxidase Activity in Rat Liver 
Microsomes Expressing CYP2E1, CYP1A1/2, 
and CYP3A1/2
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A characteristic of microsomal CYP enzymes is their ability to generate H2O2, 
either directly or indirectly via superoxide anion, a reaction referred to as 
"NADPH oxidase" activity. Excessive H2O2 production by CYPs can lead to 
the accumulation of additional cytotoxic reactive oxygen species and these 
can compromise cellular functioning and contribute to tissue injury. There 
is a widespread view that CYP2E1 is the major enzyme in microsomes generat-
ing H2O2 and that this activity is not related to its monooxygenase activity. 
Because of this property, CYP2E1 is thought to play a major role in oxida-
tive stress. Conversely, we found that CYP2E1-enriched isoniazide-induced 
rat liver microsomes produce approximately the same amounts of H2O2 (4-5 
nmol/min/mg of microsomal protein) as control microsomes (4-5 nmol/min/ 
mg protein) and CYP1A2 enriched β-naphthoflavone induced microsomes 
(3-4 nmol/min/mg protein), and much less than CYP3A1/2 enriched dexta-
methasone-induced microsomes (12-16 nmol/min/mg protein). To analyze the 
relative contribution of these CYPs to NADPH oxidase activities, we used 
form selective CYP inhibitors of monoxygenase activity including 4-methyl-
ypyrazole, α-naphthoflavone and ketoconazole for CYP2E1, CYP1A2, and 
CYP3A1/2, respectively. These inhibitors were found to block both mono-
oxigenase activities and H2O2 generation by recombinant CYP2E1, CYP1A1/2, 
and CYP3A1/2, respectively. In isoniazide-induced rat liver microsomes, 
while readily inhibiting p-nitrophenol hydroxylation, 4-methylpyrazole did 
not inhibit NADPH oxidase activity. In contrast, α-naphthoflavone and ke-
toconazole, while inhibiting ethoxyresorufin demethylation and alprazolam 
hydroxylation in β-naphthoflavone- and dexamethasone-induced rat liver 
microsomes, respectively, also inhibited the generation of H2O2 by these mi-
crosomes. These data demonstrate that CYP2E1 does not possess the great-
est NADPH oxidase activity. The fact that H2O2 production was inhibited 
by α-naphthoflavone in β-naphthoflavone induced microsomes and keto-
conazole in dexamethasone-induced microsomes, but not 4-methylpyrazole in 
isoniazide-induced microsomes suggests that CYP1A1/2 and CYP3A1/2 and 
possibly other CYPs, but not CYP2E1, are predominantly responsible for H2O2 
generation in microsomal membranes. Supported by NIH grants AR055073, 
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3097 Comparative Analysis of 10 CYP2A6 from 
Different Sources by Assessing Nicotine 
C-Oxidation Activities
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Nicotine is one of the major addictive components in tobacco. It is demon-
strated that the rate of elimination of nicotine can influence self-determined 
consumption in humans. The primary catalyst of nicotine metabolism in 
humans is CYP2A6, which is a member of the cytochrome P450 (P450) fam-
ily and one of the enzymes responsible for the metabolism of therapeutic 
drugs, such as nicotine, 4-(methylthio)anisole-1-(3-pyridyl)-1-butanone, and 
N-nitrosodimethylamine. CYP2A6 proteins showed significant individual 
differences in the metabolic effect of nicotine, which were associated with 
individual variation in smoking behavior, drug toxicities, and the risk of de-
veloping several cancers. In this study, our goals were to explore the relation-
ship between the expression levels and enzymatic activities of 10 CYP2A6 
proteins which were heterologously expressed in different sources, including 
recombinase, human cells and liver microsomes of humans, beagles, rhesus 
macques and mouse. The enzymatic activities were assessed on the basis of 
icotine C-oxidation by LC-MS/MS, and the CYP2A6 content were determined 
by western blot assay. The results showed that, among the 10 CYP2A6 from 
different sources, 25-donor mixed gender pooled human liver microsomes, 
human CYP2A6 expressed in Escherichia coli and liver microsome of male rhes-
sus macaques exhibited markedly reduced activity toward nicotine, whereas 
7 others exhibited no enzymatic activity. Meanwhile, there was a positive cor-
relation between the content of CYP2A6 and the activity of it approximately.
This study would significantly enrich our knowledge about the metabolism of nicotine enzyme by CYP2A6, and also provide useful insight into the toxicological evaluation of cigarette smoke in vitro.

The Role of Hepatic Cytochrome P450 in the Cytotoxicity of Dronedarone

Dronedarone is used to treat patients with cardiac arrhythmias and has been reported to be associated with liver injury. Our previous mechanistic work demonstrated that DNA damage-induced apoptosis contributes to the cytotoxicity of dronedarone. In this study, we examined further the underlying mechanisms and found that after a 24-h treatment of HepG2 cells, dronedarone caused cytotoxicity, G1-phase cell cycle arrest, suppression of topoisomerase II, and DNA damage in a concentration-dependent manner. We also investigated the role of cytochrome P450s (CYPs)-mediated metabolism in the dronedarone-induced toxicity using our previously established HepG2 cell lines expressing individually 14 human CYPs (1A1, 1A2, 1B1, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, 3A5, and 3A7). We demonstrated that CYP3A4, 3A5, and 2D6 were the major enzymes that metabolize dronedarone, and that CYP3A7, 2E1, 2C19, 2C18, 1A1, and 2B6 also metabolize dronedarone, but to a lesser extent. Our data showed that the cytotoxicity of dronedarone was decreased in CYP3A4-, 3A5-, or 2D6-overexpressing cells compared to the control HepG2 cells, indicating that the parent dronedarone has higher potency than the metabolites to induce cytotoxicity in these cells. In contrast, cytotoxicity was increased in CYP1A1-overexpressing cells, demonstrating that CYP1A1 exerts an opposite effect in dronedarone’s toxicity, compared to CYP3A4, 3A5, or 2D6. We also studied the involvement of topoisomerase II in dronedarone-induced toxicity, and demonstrated that the overexpression of topoisomerase II caused an increase in cell viability and a decrease in H2AX induction, suggesting that suppression of topoisomerase II may be one of the mechanisms involved in dronedarone-induced liver toxicity.

Effect of the CYP2C8*3 Variant on Asthma Symptom Control and Montelukast Efficacy
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Asthma causes chronic inflammation of the airways and bronchial hyper-reactivity. Despite being treated with appropriate therapeutics, many asthmatics experience suboptimal control of their symptoms. This may be due in part to the effect of genetic variations in genes that dictate drug disposition and/or response. We previously found an association between CYP3A4*22 and CYP3A5*3 with improved asthma control among individuals being treated with fluticasone propionate (FP) and beclomethasone dipropionate (BDP), respectively. We hypothesized that additional genotype-phenotype correlations may exist for asthma drug metabolism pathways. Using TaqMan Open Array technology we assayed 170 different single nucleotide polymorphisms in approximately 1500 genomic DNA samples collected from children undergoing treatment for asthma. Multiple SNPs in the CYP3A gene family, as well as other cytochrome P450 enzymes, P450 reductase, the glucocorticoid receptor, and other asthma-related genes were assayed. We found a new association between variation in CYP2C8 and asthma control scores. For the CYP2C8*3 variant, which is defined by amino acid substitutions at Arg139(Lys) and Lys399(Arg), the mean asthma control scores were lower (i.e., better asthma symptom control) in patients expressing ≥1 copy of CYP2C8*3 allele, when compared to patients with the wild-type CYP2C8*1/*1 genotype (4.28 [n=845] vs. 3.32 [n=172]). Furthermore, when results were stratified by treatment with Montelukast, patients with ≥1 copy of CYP2C8*3 exhibited lower mean asthma control scores (3.56 [n=55] vs. 5.44 [n=214] [p=0.0017]), an effect that was not observed for several other asthma controller medications. Cytochrome P450 (CYP) 2C8 is the principal enzyme involved in the metabolism of Montelukast. Efforts are underway to study the role of CYP2C8 variation in Montelukast clearance and efficacy in human lung cells. These, and future findings should further our long-term goal of improving treatment of asthma through better understanding of the mechanisms associated with sub-optimal clinical responses to current therapies.

Effects of CYP1A on Benzo[a]pyrene and a Complex Polycyclic Aromatic Hydrocarbon (PAH) Mixture Exposure in Caenorhabditis elegans
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Polycyclic aromatic hydrocarbons (PAHs) are environmental toxics produced during incomplete combustion; many are known carcinogens. The Elizabeth River in southeastern Virginia was declared a Superfund site due to coal tar creosote contamination that resulted in complex mixtures of high molecular PAHs accumulating within the water and sediment. PAHs are metabolized via cytochrome P450 enzymes (P450s, CYP for specific isoforms), mainly of the CYP1 family. CYP1A1 in human P450 metabolism, a carcinogenic metabolite can be created, which has the potential to damage cellular macromolecules such as DNA and proteins. Thus, P450s have the potential to eliminate PAHs and, paradoxically, create carcinogenic metabolites, so the question of whether CYP1A activity is beneficial or deleterious is unclear. We designed experiments aimed at answering this question using transgenic CYP1A-expressing Caenorhabditis elegans with a special focus on mitochondrial function. Mitochondria may be particularly sensitive to PAH exposure because the mitochondrial membrane attracts lipophilic molecules such as PAHs, and mitochondria lack the nucleotide excision repair pathway, which is primary repair pathway responsible for removing DNA adducts caused by PAH metabolites. In this study, we exposed wild-type and transgenic zebrafish CYP1A-expressing. C. elegans nematodes to a complex PAH mixture, the Elizabeth River Sediment Extract (ERSE), and a well-studied PAH found in the mixture, benzo[a]pyrene (BaP). The effect of CYP1A on BaP and ERSE exposure was tested through ATP, growth, reproduction, and DNA damage assays. CYP1A provided significant (p<0.05) protection against Benzo[a]pyrene-induced growth delay, DNA damage, reduction of offspring, and reduction of steady-state ATP levels. The protective effects of CYP1A on ERSE-dosed nematodes were less dramatic than those seen in BaP-dosed nematodes. CYP1A protected against ERSE-induced reduction of steady state ATP levels at all doses of ERSE studied, but only protected against growth delay and DNA damage at low ERSE exposures. There was no evidence of increased DNA damage due to the carcinogenic metabolite at high doses of ERSE. Thus, in this study, we found CYP1A activity to be mostly protective and hypothesize that another cytochrome P450 isoform such as CYP1B may be mainly responsible for producing the genotoxic metabolite.
Hypersensitivity to Cisplatin and Gentamycin Induced Nephrotoxicity in Mice with Decreased Expression of the NAPDHCytochrome P450 Reductase

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Cytochrome P450 reductase (CPR or POR) is an obligatory redox partner for all microsomal P450 enzymes. A large, global decrease in POR expression, in a Cpr-low mouse model, was previously found to lead to altered sex steroid hormone homeostasis, female infertility, and decreased capacity for xenobiotic metabolism. The aim of this study was to further characterize the Cpr-low mouse model, in order to identify additional phenotypes that may reveal vulnerability in people with low POR expression. Here, we report differences between wild-type and Cpr-low mice in their sensitivities to drug-induced toxicity in the renal proximal tubules. We studied two drugs, the anticancer drug cisplatin and the antibiotic drug gentamycin, which are known as renal toxicants and do not P450 substrates. Toxicity was assessed by blood urea nitrogen (BUN) levels and histopathology of the kidney. At drug doses that do not cause renal toxicity in wild-type mice, cisplatin caused significant increases in BUN levels over vehicle control group in both male and female Cpr-low mice, whereas gentamycin caused significant increases in BUN levels over vehicle control group in male but not female Cpr-low mice. Corresponding histopathological changes, including appearance of extensive proximal tubule vacuolization, were found in the kidneys of drug-treated Cpr-low mice. These findings suggest that POR expression level could be a risk factor for drug-induced nephrotoxicity in patients.

Comparative Analysis of Epigenetic and Gene Expression Levels Using hGFP1A1 Transgenic Mice with Different Mouse Strains Exposed to 3-Methyloctanol

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Lung cancer is the leading cause of death worldwide. It is known that the exposure of humans to polycyclic aromatic hydrocarbons (PAHs) via cigarette smoking or by consumption of charcoal broiled steaks is associated with an increased risk of lung cancer. In this study, we tested the hypothesis that the PAH, 3-methyloctanol (MC) elicits persistent human CYP1A1 induction in lungs and livers of transgenic hGFP1A1 mice in strain-specific manner, and CYP1A2 plays a mechanistic role in this phenomenon. WT, hGFP1A1, hGFP1A1/ Cyp1a1-l null or hGFP1A1/Cyp1a2-null on A/J or C57BL/6J background were divided into two groups. Group I was treated with vehicle corn oil (CO) (8 ml/kg) and group II was treated with four doses of MC (100 µmol/kg) once daily for 4 days in C57BL/6, or with single dose of MC (40 µmol/kg) by i.p in A/J mice due to differences in the susceptibility to develop tumors. Four animals in each group were sacrificed at 1, 8 and 15 days after MC withdrawal, lung and liver tissues were collected. The differences in gene expression of mouse and human mRNA, protein content and enzyme activities of CYP1A1 and 1A2, epigenetic markers and miRNA levels were determined at all time points. Though there was a similarity in mouse and human CYP1A1, and mCYP1A2 mRNA, protein levels and enzyme activities persisted up to 8 days and declined by 15 days, there were significant differences in the epigenetic markers and mRNA levels at various time points in a strain-dependent manner. These results suggested that the human CYP1A1 as well as host CYP1A1 and 1A2 enzymes contribute to the differential epigenetic and miRNA expression or vice versa in mice in relation to PAH-mediated carcinogenesis. The current comparative study may be useful to elucidate the role of genetic and epigenetic factors responsible for the differences in the susceptibilities to develop cancers in humans.

The Emerging Contaminant 3,3’-Dichlorobiphenyl (PCB-11) Impedes AhR Activation and Cyp1a Activity to Modify Embryotoxicity of AhR Ligands in the Zebrafish Embryo Model (Danio rerio)

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3,3’-dichlorobiphenyl (PCB-11) is a non-legacy lower-chlorinated PCB congeners that is a byproduct of pigment manufacturing. It is widely detected in environmental samples and has been detected in human serum, but its toxicity potential is poorly understood. We assessed its embryotoxicity and interactions with the aryl hydrocarbon receptor (AhR) pathway in developing zebrafish (Danio rerio). Zebrafish embryos were exposed to 0.2 µM, 2 µM, or 20 µM PCB-11 from 24-96 hours post fertilization (hpf), when they were assessed for gross morphology and Cyp1a activity using the in vivo EROD bioassay. AhR pathway interactions were probed by co-exposing the zebrafish to the AhR agonists PCB-126 or the model PAH beta-naphthoflavone (BNF). Liver development was assessed using the Tg(gut:GFP) zebrafish line. Zebrafish exposed to 20 µM PCB-11 for 4 days and 20 µM GFP-PCB or 20 µM BNF for 4 days were collected at 60 hpf for qRT-PCR and histology. Zebrafish exposure to PCB-11 alone mildly affected EROD activity but did not affect gross morphology. However, 20 µM PCB-11 alone altered the transcription of xenobiotic metabolism and liver development genes, impeded liver development, and increased vacuole formation. In co-exposures, 20 µM PCB-11 induced deformation caused by PCB-126 but exacerbated deformities in co-exposures with BNF. The 20 µM PCB-11 concentration tested in zebrafish can affect liver development, act as both a partial agonist/antagonist of the AhR pathway, and act as an antagonist of Cyp1a activity to modify the toxicity of compounds that interact with the AhR pathway.

Effects of Four Marine Toxins: Ciguatoxin, Maitotoxin, Brevetoxin, and Saxitoxin on Mouse Liver Detoxification Enzymes

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Dinoflagellates form a large group of flagellate organisms that are a predominant part of the plankton of aquatic systems. A number of illnesses have been traced to the ingestion of toxins produced by dinoflagellates. The transfer of these toxins through the food chain are eventually consumed by humans and are the cause for many fish poisoning diseases such as ciguatera, paralytic and neurotoxic shellfish poisonings. The metabolism of marine toxins are fairly understood, but that of others such as Ciguatoxin-1 (CTX-1), Maitotoxin-2, Brevetoxin-2 (PbTx-2) and Saxitoxin (STX) remain poorly understood despite being extensively studied with respect to their biological structure and activity. This study evaluated the effects of four purified marine toxins: Ciguatoxin (CTX-1), Maitotoxin (MTX-2), Saxitoxin (STX) and Brevetoxin (BTX-2) on murine (C57BL/6) and Webster mice liver detoxification enzymes. MTX-2 preferentially reduced the expression of hepatic cytochrome P4503A2 protein and mRNA levels. Histopathological analyses of the livers of control and experimental animals showed perivenous and midzonal necrosis in mice treated with MTX and CTX. Sublethal doses of MTX produces liver necrosis and affects the concentration of liver detoxification enzymes in particular cytochrome P4503A2. While MTX and CTX exerted similar effects on detoxification enzymes, BTX and STX were found to induce cytochrome P4501A2 and to a lesser extent cytochrome P4503A2 at the concentrations tested. To the best of our knowledge, these are the first known studies to evaluate the effects of these marine toxins on hepatic detoxification enzymes in mammals and the first to demonstrate their hepatotoxicity in mice.

Effect of Lambda-Cyhalothrin on the Transcription of the Genes Related to CYP Isomorphs and Inflammation, Oxidative Stress, and Apoptosis Responses in the Liver


Lambda-cyhalothrin, a type II 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. The aim of this work was to evaluate potential effects of lambda-cyhalothrin on the transcription of genes related to CYP isomorphs and inflammation, oxidative stress and apoptosis responses in the liver. All experimental procedures involving animals were conducted in accordance with the ethics requirements and authorized by the Institutional Animal Care and Use Committee of the Complutense University. Male Wistar rats received single daily oral administration of lambda-cyhalothrin at doses levels of 4 and 8 mg/kg bw, for 6 days. Animals were euthanized by decapitation after the last administration, livers excised, and portions of each liver tissue sample were individually stored at -80°C until gene expression analysis. Quantitative real-time PCR assays for rat CYP1A1/1A2, CYP2A1, CYP2B1/2B2, CYP2E1, CYP3A1/3A2, and CYP4A1 mRNA as well as for rat IL-1β, NFκB, Nrf2, p53, Casp-3, Bax and Bcl2 mRNA were performed to analyze mRNA gene expressions. The results included: (1) in both treatment groups, a significant increase of CYP isoform mRNA levels, mainly CYP2B1 (1463% and 961%) and CYP2B2 (604% and 501%); (2) IL-1β, Casp-3 and Bcl mRNA levels increased significantly in the group treated 4
mg/kg bw, 6 days. (19 %, 29 % and 46 %); (3) a significant induction of NfE8 and p53 mRNA was observed in both groups of treatment (37 %, 18 % and 55 %, 33 %, respectively); (4) NfE2 mRNA was induced (30 %) only at dose of 8 mg lambda-cyhalothrin/kg bw, for 6 days. These results suggest that an indiscriminate use of the pyrethroid lambda-cyhalothrin may lead to hepatic injuries in a time- and concentration-dependent manner. The potential of the pyrethroid lambda-cyhalothrin to induce hepatic aromatic-metabolizing enyzmes should be considered for the human risk assessment of this pyrethroid. Work supported by Project Ref. RTA2015-00010-C03-03 from Ministerio de Economía, Industria y Competitividad, Spain.

3108 Evolutionary Patterns in Expression of UDP-Glucuronosyltransferases in Vertebrates

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UDP-glucuronosyltransferases are important detoxification enzymes in a wide variety of species. However, over the last decades, more and more examples have been discovered of perplexing differences in the expression of phase 2 detoxification enzymes in vertebrate species, not only in UDP-glucuronosyltransferases but also in glutathione S-transferases. These enzymes are involved in conjugating a hydrophilic group to a poorly water soluble substrate, thus reducing the biological activity or toxicity of the substrate, and facilitating the excretion of the conjugated complex. To further investigate species differences in marine fish taxa, a variety of species was evaluated for the activity of sulfation and glucuronidation enzymes, using the natural hormone 178-estradiol and the environmental toxicant 9-hydroxy-benz(a)pyrene as substrates. Primitive fish species like hagfish and lamprey appeared to have no glucuronidation activity towards these substrates, while activity in sharks and rays was lower than in teleost fishes. This would indicate that the earliest vertebrates had no glucuronidation capacity, and that the array of glucuronosyltransferases that is known in modern fish species and other vertebrates has evolved later. However, there are dramatic examples of other vertebrates that don’t have glucuronidation capability towards phe-nolic substrates. We performed experiments to demonstrate that a number of snake species have phenol-type glucuronidation activity, which is essential, like phenol-type glucuronosyltransferases in obligate carnivores and other reptilians like turtles and alligators had much lower activity than mammals. The lack of phenol-type glucuronidation enzymes, combined with the lack of N-acetylsulfotransferase, leads to an accumulation of highly toxic aminophenol in snakes when exposed to acetoaminophen, which phenomenon is used to control the invasive brown tree snake in Guam. In addition, it has been known that the felines lack phenol-type glucuronida-tion activity, which makes them extremely sensitive to compounds like acetaminophen. The explanation for this diverse expression of glucuronosyltransferases in different vertebrate taxa is probably a combination of phylogenetic origins and the degradation of genomic information in genes that are not essential, like phenol-type glucuronosyltransferases in obligate carnivores like cats, snakes and alligators.

3109 Absorption, Distribution, Metabolism, and Excretion of Didecyl Dimethyl Ammonium Chloride (DDAC) in Rats

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The absorption, distribution, metabolism, and excretion (ADME) profiles of DDAC were evaluated in Good Laboratory Practice and regulatory guideline compliant studies. In definitive experiments, ADME profiles of [14C]-DDAC were evaluated in Sprague-Dawley rats (5/sex/group) following a single low dose (10 mg/kg) or a single high dose (50 mg/kg) via oral gavage, after a single intravenous (i.v.) dose (10 mg/kg), or after repeated oral doses. In the repeated dose ADME experiment, rats were fed diets containing 100 ppm ADBAC for 14 days prior to a single oral gavage dose of 10 mg/kg [14C]- ADBAC. Following [14C]-ADBAC dosing, urine and feces were collected periodically and 7 days after dosing the rats were euthanized and selected tissues and organs harvested. Total radioactivity recovery for all groups ranged from 98.4-108% for males and 94.6-111% for females. In oral experiments, approximately 87-99% of the recovered radioactivity was found in the feces, 5-8% in the urine, and <1% in tissues. No significant differences in ADE patterns were noted between males and females of all orally dosed groups. Following acute i.v. administration of [14C]-ADBAC, approximately 45-55% of the administered dose was found in feces, 20-30% in urine, and 30-35% in tissues. The large fraction of [14C]-ADBAC present in the urine and feces following i.v. administra-tion indicates that ADBAC is excreted by the kidney and liver. Preliminary experiments with acute oral administration indicated little or no radioactivity as [14C]-CO2 was eliminated in the expired air and minimal radioactivity was systemically absorbed; however, detectable levels of radioactivity were pres-ent in the blood within 15 minutes. Appropriative extraction, chromatographic, and spectral techniques were used to identify major metabolites (>10%) of ADBAC in the feces following oral administration of [14C]-ADBAC at 5 mg/kg. Approximately 60-70% of the recovered radioactivity was ADBAC. Spectral analysis of the metabolites demonstrated that oxidation of the alkyl acid side chain and the terminal carbon to form hydroxyl ketone compounds also occurs, resulting in more polar and thus, more readily soluble metabolites for urinary elimination. There was no evidence of modification on the benzyl or N-methyl substituent.

3110 Absorption, Distribution, Metabolism, and Excretion of Alkyl Dimethyl Benzyl Ammonium Chloride (ADBAC) in Rats

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The absorption, distribution, metabolism, and excretion (ADME) profiles of ADBAC were evaluated in Good Laboratory Practice and regulatory guideline compliant studies. In definitive experiments, ADME profiles of [14C]-ADBAC were evaluated in Sprague-Dawley rats (5/sex/group) following a single low dose (10 mg/kg) or a single high dose (50 mg/kg) via oral gavage, after a single intravenous (i.v.) dose (10 mg/kg), or after repeated oral doses. In the repeated dose ADME experiment, rats were fed diets containing 100 ppm ADBAC for 14 days prior to a single oral gavage dose of 10 mg/kg [14C]- ADBAC. Following [14C]-ADBAC dosing, urine and feces were collected periodically and 7 days after dosing the rats were euthanized and selected tissues and organs harvested. Total radioactivity recovery for all groups ranged from 98.4-108% for males and 94.6-111% for females. In oral experiments, approximately 87-99% of the recovered radioactivity was found in the feces, 5-8% in the urine, and <1% in tissues. No significant differences in ADE patterns were noted between males and females of all orally dosed groups. Following acute i.v. administration of [14C]-ADBAC, approximately 45-55% of the administered dose was found in feces, 20-30% in urine, and 30-35% in tissues. The large fraction of [14C]-ADBAC present in the urine and feces following i.v. administra-tion indicates that ADBAC is excreted by the kidney and liver. Preliminary experiments with acute oral administration indicated little or no radioactivity as [14C]-CO2 was eliminated in the expired air and minimal radioactivity was systemically absorbed; however, detectable levels of radioactivity were pres-ent in the blood within 15 minutes. Appropriative extraction, chromatographic, and spectral techniques were used to identify major metabolites (>10%) of ADBAC in the feces following oral administration of [14C]-ADBAC at 5 mg/kg. Approximately 60-70% of the recovered radioactivity was ADBAC. Spectral analysis of the metabolites demonstrated that oxidation of the alkyl acid side chain and the terminal carbon to form hydroxyl ketone compounds also occurs, resulting in more polar and thus, more readily soluble metabolites for urinary elimination. There was no evidence of modification on the benzyl or N-methyl substituent.

3111 Metabolite Profiling and Identification in Dog Plasma and Tissues of Edasalonexent (CAT-1004), a Conjugate of Docosahexaenoic Acid (DHA) and Salicylic Acid (SA) Using Smart Linker Technology

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Edasalonexent (CAT-1004) is an oral small molecule which inhibits NF-kB, a transcription factor that is activated in Duchenne Muscular Dystrophy (DMD) and drives inflammation, fibrosis, and muscle degeneration. Edasalonexent (CAT-1004), a Conjugate of Docosahexaenoic Acid (DHA) and Salicylic Acid (SA) Using Smart Linker Technology. This methodology allows the simultaneous intracellular delivery of two bioactives to elicit a pharmacological response that could not be elicited by administering the bioactives separately or in combination. Edasalonexent is currently in clinical development in 4- to 7-year-old boys with DMD regardless of mutation. The present study was conducted to identify edasalonexent metabolites formed in beagle dogs in vivo and to determine their levels in plasma and distribution in heart, liver and skeletal muscle using liquid chromatography-mass spectrometry (LC-MS). Beagle dogs were treated with 1,000 mg/Kg/day for 39 consecutive weeks. No treat-ment-related signs of toxicity were detected in this study. Edasalonexent was bio-transformed into thirteen and ten metabolites in male and female dogs, respectively; the biotransformation observed included phase I (oxidation and hydrolysis) and Phase II (mainly glucurono-conjugation) pathways with large overlaps between the two genders. Among the tissues analyzed (plasma,
heart, liver and skeletal muscle), liver showed the presence of the highest number and levels of edasalonexent metabolites. In heart, liver and skeletal muscle of both male and female dogs, DHA represented between 80 and 99% of the total drug related material detected (as relative LC/MS peak area intensity). In addition, consistent with the known metabolism of unsaturated fatty acids, oxidative metabolites (in the DHA moiety) were also identified. These results suggest that after extravasation of edasalonexent to beagle dogs DHA is preferentially retained by heart and skeletal muscle, two affected tissues in DMD disease, more than the parent compound and/or any other metabolite detected in the same tissues. DHA was also one of the major circulating metabolites observed in dogs.

2,4,6-Tribromophenol (TBP) is naturally-occurring bromanophenol that is widely used as a brominated flame retardant and wood antifungal. TBP is found in soil, dust, wild-caught seafood, and humans. TBP in vivo studies of oral and dermal disposition were conducted in rats and mice. Human dermal disposition was estimated based on studies of ex vivo split-thickness human and rat skin. Following intravenous administration, TBP was rapidly excreted in urine, with 89-94% recovered in urine, 5% in feces, and 1-2% in blood/tissues after 24h. TBP administered to female SD rats (0.1-1000 µmol/kg) by gavage was well absorbed, with ~88% eliminated via urine after 24h, 11% in bile, and 3% in feces. Male and female SD rats and B6C3F1/J mice had similar disposition profiles when administered a single oral dose of TBP (10 µmol/kg), with urine and fecal recoveries varying only slightly by sex or species. TBP kinetics were linear across a range of doses in short-term studies (0.5-10 µmol/kg, 4 h). Urine contained TBP, TBP-glucuronide, and TBP-sulfate. Bile contained TBP-glucuronide while fecal extracts contained only parent TBP. TBP did not appear to bioaccumulate after single or 5 repeated oral administrations. TBP was readily absorbed at all doses and routes tested. Oral bioavailability was 23-27%. TBP readily passed unchanged through both human and rat skin with 49% predicted to be dermally bioavailable in humans. We conclude from these data that humans are likely to have significant systemic exposure when TBP is encountered in the workplace, home, or outside environment. This research was supported in part by the Intramural Research Program of NIH/NCI [Project ZIA BC 011476]. This abstract does not necessarily represent US EPA policy.

Pharmacokinetics of TBP (CAS No. 118-79-6) was assessed in male Sprague-Dawley rats and compared to emtricitabine free macromolecular prodrug platform may have extraordinary potential for HIV and other anti-viral therapies. Funded by NCI Contract No. HHSN26120080001E, and supported by the National Cancer Institute of North Carolina at Chapel Hill Center for AIDS Research (CFAR), an NIH funded program P30 AI05410.

1,3-Dichloropropene (1,3-D) showed a statistically increased incidence of bronchoalveolar adenomas of in male B6C3F1 mice at 60 ppm during chronic inhalation testing. Saturation of metabolic clearance at this level would lack relevance for human health risk assessment. Therefore, the linearity of 1,3-D concentrations in mouse blood was investigated on Day 15 of repeated nose-only inhalation exposure to 0, 10, 20, 40, 60, and 120 ppm (6 h/d, 7/d/ wk). Additional groups were included at 20, 60 and 120 ppm for blood collection at 1.5-3 h of exposure to determine AUC and t½. As exposure increased from 10 to 120 ppm, mean steady-state blood concentrations ranged from 0.968 to 63.3, 7.19 to 283, and 8.16 to 346 ng/g blood for cis, trans and total (cis+trans) isomers of 1,3-D, respectively. The time-course showed steady-state blood concentrations at 1.5 and 3 h at 20 and 60 ppm, and by 6 h at 120 ppm. Blood concentrations declined rapidly after exposure (t½ = 5 to 12 min). AUC estimates for total 1,3-D were not dose-proportional, with values of 4.4, 30.2 and 89.7 min·µg/g, at the 20, 60 and 120 ppm, respectively. Statistical analysis for dose proportionality included a second-order regression model and a robust, piecewise linear (hockey-stick) model. Arithmetic comparisons for linearity were made at the 20, 60 and 120 ppm. Blood concentrations in blood were compared to the 10-fold (+) threshold of 60 ppm. These results support non-relevance of 1,3-D induced benign pulmonary tumorigenicity in mice for human health risk assessment.
3116 Comparison of Plasma Pharmacokinetics of Intravenous and Intramuscular Administration of Midazolam in Male Sprague-Dawley Rats

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Benzodiazepines are commonly used as first-line therapy to treat acute seizures and status epilepticus (SE), characterized by prolonged seizure activity, which if untreated leads to severe brain pathology. The benzodiazepine midazolam is a positive allosteric modulator of the GABA receptor and potentiates GABAergic inhibition. Midazolam has been sought to replace diazepam to treat seizures induced by chemical warfare nerve agents (CWA) for its rapid absorption and short duration of action. Although midazolam is often administered intravenously in the clinic, intramuscular administration would enable rapid treatment in the event of a high number of casualties. A New Drug Application for the use of intramuscular midazolam to treat SE was recently approved by the US FDA. In the present study, we compare the plasma pharmacokinetics of intravenous and intramuscular midazolam administration in rats to determine how route of administration affects the absorption rate of midazolam in rats, male rats pre-implanted with jugular catheters for repeated blood draws received midazolam (1 mg/kg) via intravenous or intramuscular injection and then had blood drawn at a range of time points (2-240 minutes) following injection. Sampling was randomized and each time point was sampled from up to six animals. Plasma was separated by centrifuging shortly after collection and frozen until biochemical assays were conducted. Samples were analyzed for peak plasma concentration using liquid chromatography and tandem mass spectrometry (LC-MS/MS). WinNonlin was used to analyze data using both non-compartmental analysis (NCA) and compartmental model fitting. Pharmacokinetic parameters were determined for intravenous (IV) and intramuscular (IM) administration including time to maximum concentration, maximum concentration, half-life of elimination, apparent volume of distribution, and area under the curve. Maximum plasma concentration (443±181 ng/mL) occurred within minutes following IM (6 minutes) and was eliminated twice as fast for IV than for IM, with the half-lives being 33 and 60 min, respectively. Bioavailability of midazolam by the IM route was approximately 50%. Volume of distribution was large being approximately 2 L/kg for IV and IM. A comparison of PK data between the two exposure routes will be presented. Funding was provided by the Defense Threat Reduction Agency.

3117 Cyfluthrin Toxicokinetics in Plasma and Nervous Tissues after Oral Administration in Rats


The intended uses of cyfluthrin, Type II pyrethroid insecticide, are to control flying and crawling insects, such as house flies, litter beetles as well as flies and red ants, cockroaches, ants and termites indoors. Cyfluthrin is acutely very toxic when administered orally in corn oil (LD₅₀ 250 mg/kg bw). Clinical signs were observed in all animals and indicated an effect on the CNS (tremor, rolling movements, disturbed motility and respiration). The aim of this work is to support new available pharmacokinetic data to be applied for cyfluthrin risk assessment. All experimental procedures involving animals were conducted in accordance with the ethics requirements and authorized by the Institutional Animal Care and Use Committee of the Complutense University of Madrid. Male Wistar rats received cyfluthrin (single oral dose 20 mg/kg bw). Serial blood and brain tissue samples were obtained and analyzed. Cyfluthrin concentrations in plasma and brain tissues (hypothalamus, striatum, hippocampus and frontal cortex) were quantified by LC/MS. Cyfluthrin disposition was best described by the use of a two-compartment open model. Plasma and nervous tissue kinetics showed an extensive oral absorption of cyfluthrin and a slow elimination. In plasma, the oral absorption half-life (T1/2a) and maximal concentration (Cmax) were 1.58 h and 0.385 µg/mL, and the elimination half-life (T1/2b) was 17.15 h. After oral administration, cyfluthrin was widely distributed to brain tissues. Cyfluthrin peak concentrations (Cmax, range 0.40 to 1.21 µg/g) were achieved in all brain regions at the similar time (Tmax, range 4.02 to 4.56 h). The primary target nervous tissue appears to be hypothalamus. Peak concentration of cyfluthrin in hypothalamus tissue (Cmax, 1.21 µg/g) was about 3.3 times higher than in plasma. Nervous tissue accumulation was also reflected by the area under the concentration-time curve ratios of tissue/plasma. The ratio AUC(0-24) tissue/AUC(0-24) plasma for cyfluthrin was 3.17 for hypothalamus. Because many toxicokinetic changes appear to have toxicokinetic basis, our results suggest that the indiscriminate use of this insecticide could induce neurological disorders after long-term. Project Ref. RITA2015-00010-C03-03 from Ministerio de Economía, Industria y Competitividad, Spain.

3118 Comparative Toxicokinetic Analysis of Available Inhalation and Oral Data on Cobalt and Its Salts

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Cobalt (Co) and its salts were reported to show carcinogenic incidences after inhalation exposure to animals and humans. However, no reports of oral carcinogenicity of Co and its salts are available. One approach for addressing this data gap is to use available inhalation data to assess the safety of Co and its salts via the oral route of exposure. However, the first step is to compare the toxicokinetics (TK) of oral and inhalation exposure for Co and its salts and determine if there are any exposure route-related differences in TK of Co and its salts. We reviewed available TK studies on Co and its salts and determined that TK profiles of different Co salts may differ due to variability in physicochemical properties of the test substances, such as solubility, salt size, particle size, etc. Overall, the oral bioavailability of soluble and insoluble Co salts is low. We located oral and intravenous rat studies on soluble Co (II) chloride and used noncompartmental TK modeling to estimate area under the curve (AUC) and determined its oral bioavailability in rats. Our analysis indicated that the oral bioavailability of Co (II) chloride in rats was low (2.41%). Furthermore, we identified sex-based differences in the TK of Co (II) chloride. Noncompartment TK analysis of published data on cyanocobalamin (i.e., vitamin B12) in human subjects demonstrated that its bioavailability was low (2.16%). We observed that Co naphthenate in rats demonstrated similar AUCs to Co (III) chloride, suggesting that its bioavailability would be low. We also reviewed TK data on cobalt (II, III) oxide and concluded that inhalation data could not be used for its safety assessment of oral exposure due to differences in reported TK parameters between the two routes. In conclusion, the oral bioavailability of evaluated Co salts is low. Furthermore, TK profiles of Co and its salts may markedly differ between the two exposure routes, possibly due to different effects of test substance-related physico-chemical properties, such as solubility. Therefore, the utilization of inhalation data to assess safety of Co and its salts after oral exposure should be performed on a case-by-case basis.

3119 Comparative Toxicokinetic Study of Three Bisphenols (BPA, BPS, and BPF) in a Sheep Pregnancy Model

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Bisphenol A (BPA), S (BPS), and F (BPF) are the most abundant bisphenols detected in humans and can cross the placental barrier. We have recently shown that in vivo exposure to BPS, but not BPA, can reduce the endocrine function of the placenta. Gestational BPS exposure has been linked to longer gestation in humans and disruptions in mammary gland development. Although BPA, and recently BPS, toxicokinetics are known, those of BPF in pregnancy remain unknown. Women in the US are exposed to over 60 chemicals at any given time, and it has been demonstrated that BPA, BPS and BPF occur as a mixture in vivo. However, there has been no direct comparison among the three bisphenols, individually or in a mixture, which was the aim of our study. Given the structural and binding affinity differences among bisphenols, we hypothesized that BPF toxicokinetics will differ to that of BPS or BPA. Fetal catheterizations were conducted in pregnant sheep (n=6) at mid-pregnancy that were injected with either a single dose of BPS (n=3, 0.5 mg/kg, s.c.), or a combination of BPA, BPS, and BPF (n=3, 0.5 mg/kg for each chemical, s.c.). Maternal and fetal blood samples were collected to develop toxicokinetic profiles from 0 to 72 h. Maternal and fetal urine, and amniotic fluid were also collected. Bisphenols were assayed by HPLC/MS/MS and toxicokinetic parameters calculated via non-compartmental analysis. Half-lives varied among bisphenols in maternal circulation; BPF having the longest half-life and BPS having the shortest.
of the shortest (BPA: 5.3±0.4 h; BPS: 3.7±0.1; BPF: 7.7±2.2). On the contrary, in fetal circulation, BPS had the longest half-life and BPF had the shortest (BPA 52.0±20.3 h; BPS: 402.2±102.6; BPF: 14.2±1.8). All bisphenols reached basal levels at 48 h in maternal plasma but were still detectable in amniotic fluid and fetal urine at 72 h, highlighting the persistence of bisphenols in the fetus. This is the first study to simultaneously compare toxicokinetic profiles for BPA, BPS, and BPF during pregnancy. We demonstrated that BPS reaches in the highest relative concentration in maternal plasma and accumulates in the fetal compartment. These observations warrant further studies into progeny outcomes following gestational BPS exposure. Funded by NIHES K22ES026208 and R01ES027863 to AV-L.

3120 In Vitro ADMET and Pharmacokinetic Screening of Novel Epac1 Inhibitors for the Treatment of Rickettsiosis


Inhibition of exchange protein directly activated by cAMP 1 (Epac1) is a novel prophylactic and/or therapeutic approach for fatal rickettsiosis. The goal of this project is to design and evaluate Epac1-specific inhibitors (ESI) with favorable in vitro absorption, distribution, metabolism, excretion, and toxicity (ADMET) and pharmacokinetic (PK) properties using a systematic approach. To support the development of Epac1 inhibitors with promising in vitro and in vivo efficacy profiles, over 35 ESI have been screened for genotoxicity in vitro Ames mutagenicity and mouse lymphoma assays. Of these, ~30 compounds were selected for in vitro parallel artificial membrane permeability assay (PAMPA), metabolic stability in human, rat, dog, and mouse liver microsomes, and inhibition of specific human liver cytochrome P450 enzymes. The prototype of this series of substituted 2-(isoaxazol-3-yl)-2-oxo-N-phenyl-aceto-hydradonyl cyanides was ESI-09 from structure-activity studies, which displayed an apparent IC50 of 10.8 µM against Epac1. ESI-09 and 11 other analogs were selected for dose-range finding and PK studies in mice and rats, based on favorable in vitro results. PK studies with ESI-09 in female C57BL/6 mice after single dose ip (0 mg/kg) and oral (10, 50, and 100 mg/kg) administration indicated excellent bioavailability of ~95%, with plasma half-life (t1/2) of ~3 hr (ip) and ~3 to 6 hr (po). Apparent volume of distribution (V) of ESI-09 was ~95 ml/kg (ip) and ~230 to 310 ml/kg (po), suggesting minimal to low extra-vascular binding of ESI-09 in mice. ESI-09 also exhibited high bioavailability of ~100% after oral administration (1 and 10 mg/kg) in male and female rats, with plasma t1/2 of ~2 to 6 hr. Two other lead drug candidates NYO173 and NYO541 with IC50 values of 4.0 and 5.1 µM, respectively, were identified with promising efficacy and ADMET profiles. PK studies in female C57BL/6 mice resulted in t1/2 of 1 to 1.5 hr (NYO173) and 2 to 3 hr (NYO541). ESI-09, NYO173, and NYO541 are currently being investigated further in more detailed rat and dog PK and toxicology studies. Supported by NIH-NIAID under Grant Award R01AI111464.

3121 Comparisons of High-Dose Test Agent Levels in Plasma and Liver Over 28 Days of Dosing

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To determine potential for accumulation and safety of high dose multiple-day oral administration of a first-in-kind proposed therapeutic for inflammatory bowel disease (IBD), 1000 mg/kg/day doses suspended in Oraplus (Pennig) were given by gavage to adult male and female rats for 28 consecutive days. The product, known as BT-11, binds Lanthionine Synthetase C-Like 2 (LANCL2), which is a therapeutic target for treatment of IBD. In these experiments, blood samples were drawn at least twice from 3 rats per sex between 7 and 28 days after initiation of dosing. In addition, liver samples from 4 rats per sex were collected after dosing (day 29) and after a 2-week recovery (day 42). In female rats, plasma concentrations on day 7 averaged 20 ng/mL plasma and rose to over 70 ng/mL plasma on days 21 and 28. This increase was statistically significant. The difference between concentrations on days 21 and 28 was not significant. Plasma samples from male rats had lower concentrations of BT-11, and rose from 9.4 at 7 days to 28.0 ng/mL on day 21. Concentrations were 13.1 ng/mL at 28 days. However, these time-related concentration changes in male rats were not significant. BT-11 concentrations in liver samples were exceedingly variable but the average was higher in males than females at 28 days (429 vs 307 ng/g liver), with concentrations similar and much lower at 42 days (average 3.5 ng/g liver). No gross or pathological evidence of toxicity was seen after administration of these high doses in samples collected at 28 days. These results suggest that BT-11 is safe when high doses are given for multiple days, and that this potential therapeutic agent did not continuously accumulate in plasma over the 28-day dosing period or store in liver after cessation of dosing. Supported by VBHRC, the Virginia Biosciences Health Research Corporation.

3122 Development and Validation of an Analytical Method for Quantification of Alpha-Pinene Oxide in Rodent Blood and Mammary Tissue by GC/MS

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Alpha-pine (AP) is a potential metabolite of AP in rodents and humans. Given its potential reactivity in tissues, establishing the extent of its formation and concentration in blood and tissues is an important component of the evaluation of the toxicity of AP. The objective of this work was to validate a method to quantitate AP and in rat and mouse blood and mammary tissue, a potential target organ in vivo, in inhaled doses by gas chromatography and mass spectrometry. Standards were prepared by adding 5 µL of AP spiked solution to 100 µL rat blood followed by 300 µL ethyl acetate containing internal standard (IS, (+) limonene oxide). Sample was vortexed for 3 min and centrifuged for 3 min. The supernatant was analyzed by GC-MS in El mode. The ions monitored were m/z 109 (AP) and 94 (IS). The method was successfully validated in male and female rat plasma and rat mammary tissue. Precision and accuracy for rates were ≤±2% for all standards at all concentrations in both matrixes (r = 0.999). The relative standard deviations (%RSD) was ≤ ±5.3% and %RSD ≤ ±7.8%. The method was also evaluated in female B6C3F1 and rat mammary tissue. Approximately 50 mg mammary tissue was homogenized with a 950 µL of water. AP was added to give concentration in the range 25-500 ng/g mammary tissue. 100 µL of homogenate was extracted with ethyl acetate and analyzed as described above for blood. Mean %RE values were ≤ ±4.6% and %RSD ≤ ±8.1%. These results demonstrate that the method is suitable for the analysis of AP in rodent blood and mammary tissues generated from toxicokinetic and toxicology studies.

3123 Assessing Delivery Efficiency of Nanoparticles to Tumors in Tumor-Bearing Mice Using a Physiologically Based Pharmacokinetic Modeling and Simulation Approach

Y. Cheng, J. Riviere, N. Monteiro-Riviere, and Z. Lin

Unique physicochemical and biological properties of engineered nanoparticles (NPs) have led to key biomedical applications for diagnosis and targeting therapy of various diseases, including cancer. However, NPs translation into clinical applications are limited partly due to low delivery efficiency to the tumor and lack of knowledge on the quantitative effects of various physicochemical factors on NP tissue/tumor distribution. The goal of this study was to determine the main factors that affect tumor delivery efficiency of NPs and to identify the relative contributions of different factors using a physiologically based pharmacokinetic (PBPK) modeling approach. A PBPK model for gold NPs in healthy mice was developed first. Then the healthy mouse model was extrapolated to include a tumor compartment to simulate the biodistribution and tumor delivery of various types of NPs. A multiple linear regression model was employed to determine the potential effects of various physicochemical characteristics, including the type of administered NPs, targeting strategies, cancer types, and tumor models on tumor delivery efficiency. The healthy mouse PBPK model well predicted tissue distributions in plasma, lungs, liver, spleen, and kidneys in healthy mice after intravenous administration of 13-nm gold NPs (R² = 0.95). This PBPK model also adequately predicted most of the tumor delivery efficiencies of various types of inorganic and or-
clonidine is approved for therapy of attention-deficit hyperactivity disorder (ADHD) that is one of the most common neurobehavioral problems mainly afflicting the children and youth between 6 and 17 years of age with a high prevalence from 2% to 18% in USA. Three sustained release buccal formulations of clonidine were prepared by 3D printing technology. They showed different dissolution profiles in phosphate buffered solutions (pH 6.8). The purpose of this portion of the work was to use physiologically-based pharmacokinetic (PBPK) modeling to predict the pharmacokinetic profiles for these formulations to assist selection of an ideal formulation with a long-term sustained release for more than 72 hours. A PBPK model of clonidine was developed using Gastroplus™ (Simulations Plus, Inc.). Model parameters used in the PBPK model were collected from literature. The physicochemical parameters not available in literature were predicted using the ADMET Predictor™ module. The clinical data for clonidine was from clinical studies published in literature which included the BAC concentration–time data of both oral and intravenous (IV) administration. The Advanced Compartmental Absorption and Transit (ACAT™) and PBPKPlus™ were used for the simulation of clonidine after IV and oral administration. The simulated pharmacokinetic (PK) profiles of clonidine were compared with observed profiles for evaluation of the model performance. The model was then used to simulate PK profiles after dosing the sustained release formulations by incorporating the in vitro dissolution data into the model. The results indicated that initial high concentrations may be observed after administration of formulation 1 and 2, due to fast initial drug release, with Tmax observed around 4 hours and then fast decrease over 72 hours. Results indicated that more sustained plasma concentration could be achieved up to 72 hours after administration of formulation 3 which showed minimum initial drug release. The predicted pharmacokinetic profiles provide the evidence for formulation and preparation process screening of the sustained release buccal formulation of clonidine. This work demonstrated that PBPK modeling could be applied to optimize formulations to achieve desired pharmacokinetic profiles.

Optimization of Metabolic Parameters Using Physiologically Based Pharmacokinetic (PBPK) Modeling and Vapor Uptake Inhalation Chloroform (CHCl3) Data in F344 Rats

PBPK models are well established frameworks used to describe administration, distribution, metabolism, and excretion (ADME) of xenobiotics. To quantify metabolism, a PBPK model for a volatil compound can be calibrated with closed chamber (i.e., vapor uptake) inhalation data. Here, we graphically highlight a component of the optimization process to illustrate a strategy for metabolic parameter estimation when using vapor uptake data. Male F344 rats were exposed in vapor uptake chambers to initial concentrations of 100, 500, 1000, and 3000 ppm CHCl3. Inhalation time course data from these experiments, in combination with optimization using a chemical specific PBPK model, were used to estimate metabolism parameters Vmax and Km. Matlab® simulation software was used to integrate the mass balance equations and to perform the optimizations. The cost function used the logarithmic transformation of the in-chamber time course data and least squares to minimize the difference between data and simulation values. The final values for Vmax and Km were 4.9 mg/hr/kg

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and 0.26 mg/L, respectively. Additionally, cost function and contour plots were used to analyze the dose dependent capacity to estimate Vmax and Km within the experimental range used. Based on the combined analysis, the best concentrations for determination of Vmax were 500 and 1000 ppm with 3000 ppm having a small contribution. Least square plots confirmed 500 ppm to be the best concentration to determine Vmax. Showing the smallest differences between experimental and simulated data. The contour plots showed narrow valleys where the optimization found the best values for Vmax at both 500 and 1000 ppm. The estimation of Km was more involved, needing data across the full range of experimental concentrations (100-3000 ppm). In summary, this work should help toxicologists interested in optimization techniques to understand the overall strategy employed when calibrating metabolic parameters in a PBPK model with inhalation data. Subjecting preliminary data to the analyses described here would guide selection of optimal exposure concentrations for more accurate estimation of Vmax and Km. This strategy could be explored for in vitro metabolism parameter optimization. This abstract does not reflect US EPA policy.

Developing Databases and Models to Integrate Toxicokinetic Data for Human Health Assessment

Toxicokinetic (TK) processes such as chemical absorption, distribution, bio-transformation and excretion (ADME) play a critical role in human and ecological hazard, exposure and risk assessment. Physiologically-based toxicokinetic (PBTK) model selection is dependent on the decision-making context and information requirements. We have developed a tiered framework of PBTK models for humans and rodents and novel TK databases and Quantitative Structure-Activity Relationship (QSAR) models to apply TK models in different contexts. The TK models are generally applicable for organic chemicals and progress from one-compartment (1-CoTK) models to more sophisticated and data intensive multi-compartment (M-CoTK) models. The M-CoTK model includes capacity for simulating the parent chemical and up to 5 metabolite products. While the 1-CoTK model does not explicitly consider metabolism, all other processes of chemical uptake and elimination are quantified and the 1-CoTK model is easier to parameterize and use. The 1-CoTK and M-CoTK models require input data that can be obtained from in vitro or in vivo
studies or predicted using in silico methods. We are addressing data gaps in biotransformation rates at multiple levels of biological organization through the development of critically evaluated in vitro and in vivo databases and the development of validated QSARs following OECD QSAR guidance. The new databases include: (i) in vitro biotransformation rates (S9, hepatocytes, microsomes) from human (n=8500 chemicals) and rodent (n=7000 chemicals) assays, and (iii) in vitro transport parameter studies (n=100 chemicals) and rodent (n=700 chemicals). We have developed five validated QSARs for predicting whole-body biotransformation half-lives. The first QSAR uses a fragment-based approach (R² = 0.89, R²-ext = 0.73, root mean square error = 0.75 log units). The other four QSARs use whole molecular descriptors (R² range = 0.77 - 0.80; R²-ext range = 0.75 - 0.79, root mean square error = 0.67 - 0.69 log units). A case example demonstrates the application of the 1-CoTK model and a biotransformation QSAR for human bioaccumulation assessment for approximately 20,000 data poor commercial chemicals highlighting the critical need for reliable biotransformation half-life data.

### 3128 Towards Harmonized Test Protocols for In Vitro Hepatic Clearance Studies

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Non-animal methods for toxicokinetics, such as in vitro hepatic clearance studies, are expected to play a crucial role in next generation chemical risk evaluation. The development of harmonized test guidelines is essential for design and execution of in vitro hepatic clearance studies. As a consequence, divergent methods are used, hampering interpretation and exchange of data and regulatory acceptance. The aim of the present study was to obtain insight in the experimental conditions of in vitro hepatic clearance studies that influence obtained clearance values. To that end, in vitro hepatic clearance data and their methodological aspects (experimental setup) obtained with rat or human hepatocytes were collected from the literature. These data show that clearance values for the majority of chemicals differ more than one order of magnitude. We estimated the systematic effect of different experimental setups on the in vitro hepatic clearance data using a random forest regression analysis with several continuous and categorical experimental settings as input and (normalised) clearance data as output. The results show that several methodological aspects (e.g. 'hepatocyte concentration' and 'culture medium') have a relatively large impact on the clearance values obtained, indicating that harmonization of these methodological aspects is required to reduce variation. Indeed, the percent coefficient of variation decreased for the majority of chemicals when clearance data were only collected from studies in which methodological aspects were harmonized (e.g. only data obtained using '1.0 - 10^3 cells/mL' or only data obtained using 'WME medium'). These insights can subsequently be used as a starting point to develop a test guideline and/ or test guideline for in vitro hepatic clearance studies based on rat or human hepatocytes.

### 3129 Cell Model for Studying Nucleoside Transporters, a Key Component of the Blood-Testis Barrier


Sertoli cells in the testis represent the principle element of the Blood-Testis Barrier (BTB). Equilibrative nucleoside transporters (ENTs) are responsible for the transport of nucleosides across the BTB. These transporters are of particular interest in studying the disposition of nucleoside reverse transcriptase inhibitors (NRTIs) in the male genital tract because of their similarity in chemical structure to nucleosides. hENT1 is located on the basal membrane and hENT2 is located on the apicolateral membrane of Sertoli cells. This study characterized the transport of a specific nucleoside, uridine, in HeLa S3 cells to better understand the roles of two ENTs, ENT1 and ENT2, in nucleoside transmembrane transport. HeLa S3 cells were grown on 96 well plates and hENT1 mediated transport. HeLa S3 cells were grown on 96 well plates and cells were incubated with increasing concentrations of unlabeled uridine (0-300μM) and approximately 20nM [3H]Uridine. Two-minute kinetics of [3H]Uridine uptake showed no difference in Jmax (16.13 and 12.26 fmol cm-2 min-1) and a three-fold difference in Km (13.28 and 35.18 μM) for hENT1 and hENT2 mediated transport, respectively. This suggests that at low concentrations, hENT1 is primarily responsible for transporting uridine in these cells. Together, these data suggest that HeLa S3 cells are an adequate model for studying the characteristics of nucleoside transporters present in the BTB. Supported by R01GM123643.

### 3130 Interaction of Organophosphate Flame Retardants with the MDR1 Efflux Transporter

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Organophosphate-containing chemicals have been used as pesticides, nerve agents, plasticizers and more recently, as flame retardants in clothing, plastics, building materials, electronics, and furniture. As the use of organophosphate flame retardants becomes more widespread, the exposure of humans also increases. Emerging data suggest organophosphate flame retardants are disruptive to the reproductive, endocrine, and nervous systems. One mechanism to reduce the toxicity of chemicals is active efflux that prevents accumulation in cells. The multidrug resistance protein 1 (MDR1) is one such efflux transporter that can remove substrates from cells using energy generated from the hydrolysis of ATP. We sought to determine whether the flame retardants tricresyl phosphate (TCP), tris(1,3-dichloroisopropyl) phosphate (TDCPP), and triphenyl phosphate (TPP) are substrates and/or inhibitors of MDR1. For this purpose, HEK293 cells expressing an empty vector or the human MDR1 gene were treated with TCP, TDCPP, and TPP for 72 h and cytotoxicity (LC50) assessed using the alamarBlue assay. The positive control MDR1 substrate doxorubicin exhibited a 4-fold increase in LC50 value in cells expressing MDR1 (LC50: 1115 nM) compared to control cells (LC50: 257 nM). By comparison, the cytotoxicity of TCP, TDCPP, and TPP were similar between control and MDR1-expressing cell lines. Likewise, neither TCP nor TPP were able to alter the efflux of the MDR1 substrate rhodamine, from MDR1-expressing cells following 0.5 h co-exposure. Taken together, these in vitro data suggest that MDR1 does not confer resistance to the cytotoxicity of organophosphate flame retardants nor do these chemicals inhibit MDR1 function. Determining whether the MDR1 transporter interacts with hazardous chemicals advances our understanding of potential mechanisms that can protect against toxicity. Supported by the SOT Intern Program, ASPET, P30ES005022, R25ES020721, and R01ES021800.

### 3131 Pharmacological Inhibition of HDAC Enzymes Up-Regulates Hepatic Efflux Transporter Expression in Mice


Efflux transporters protect hepatocytes by exporting environmental toxicants, drugs, and xenobiotics into the circulation or bile. In the liver, efflux pumps include the multidrug resistance protein 1 (MDR1), breast cancer resistance protein (BCRP), and isogenous forms of the multidrug resistance-associated protein (Mrp) family. Recent research has demonstrated that histone deacetylase (HDAC) inhibitors that enhance histone acetylation can alter the expression and function of efflux transporters in cancer cells. Currently, the ability of epigenetic pathways to regulate the expression of hepatic efflux transporters is unknown. In our study, we sought to investigate whether the pharmacological inhibition of HDAC enzymes could alter efflux transporter mRNA and protein expression in mouse livers. For this purpose, adult male C57BL6 mice (N=5) were administered once daily intraperitoneal injections of vehicle or the HDAC inhibitor apicidin (5mg/kg). After 7 days, livers were collected for qPCR and western blotting. Compared to vehicle-treated control mice, treatment with apicidin reduced the hepatic mRNA expression of Mrp2 by 22% and induced Mdr1a, Mdrp3, and Mrp4 mRNAs by 25%, 64% and 131%, respectively. Likewise, the livers of mice treated with apicidin exhibited enhanced histone H3 acetylation (K9/K14) and up-regulation of Mdr1 (42%) and Mrp3 (72%) proteins. To identify candidate regulatory pathways responsible for altering transporter expression, target genes of prototypical transcription factors were quantified. Treatment with apicidin increased the hepatic expression of target genes of Car (Cyp2b10, 162%), Pxr (Cyp3a11, 113%), and Ppar alpha (Cyp4a14, 223%). Notably, no changes were observed for target genes of AhR (Cyp1a1) or Nr3f2 (Nrf1). Taken together, these data demonstrate that the pharmacologic inhibition of HDAC enzymes and increased acetylation of histone H3 proteins differentially alter the expression of hepatic efflux transporters. Supported by the Rutgers Summer Undergraduate Research Fellowship, Predoctoral Fellowship from Bristol-Myers Squibb, P30 ES005022 and ROI ES021800.
Enhanced Histone Acetylation and Renal Efflux Transporter Expression in Mice Treated with Histone Deacetylase Inhibitors


Multidrug Resistance Protein 1 (MDR1, ABCB1) and Breast Cancer Resistance Protein (BCRP, ABCG2) are key efflux transporters that regulate the disposition and toxicity of chemicals. In the kidneys, MDR1 and BCRP mediate the apical secretion of drugs and other xenobiotics from proximal tubules into urine. The purpose of the current study was to determine whether pharmacological inhibition of HDAC enzymes alters the renal expression of Mdr1 and Bcrp. Adult, male C57BL/6 mice were treated with vehicle or one of two HDAC inhibitors, suberoylanilide hydroxamic acid (SAHA) (15 mg/kg/d ip) or apicidin (5 mg/kg/d ip), daily for 7 days prior to collection of kidneys and analysis of Mdr1 and Bcrp expression by SDS-PAGE and western blotting. Treatment of mice with SAHA and apicidin increased renal acetylated histone H3 levels, confirming the pharmacological inhibition of HDAC enzymes. Compared to vehicle-treated controls, the kidneys of mice treated with either HDAC inhibitor had increased Mdr1 and Bcrp protein expression between 25 to 30%. In summary, pharmacological inhibition of HDAC enzymes increases histone acetylation and up-regulates Mdr1 and Bcrp expression which may enhance the renal secretion of drugs and xenobiotics. Supported by the Rutgers Summer Undergraduate Research Fellowship, a Predoctoral Fellowship from Bristol-Myers Squibb, P30ES050522, and R01ES021800.

Brain Region-Specific Histone Acetylation and Up-Regulation of Mdr1 and Bcrp Transporters in Mice

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The multidrug resistance protein 1 (Mdr1, Abcb1) and breast cancer resistance protein (Bcrp, Abcg2) regulate the passage of endo- and xenobiotics to protect various cells in the brain. We have previously demonstrated that histone deacetylase (HDAC) inhibitors, which are widely investigated as treatments for several brain disorders, can enhance histone H3 acetylation and regulate the expression of MDR1 and BCRP transporters in an in vitro model of the human BBB. In our present study, we sought to evaluate the ability of HDAC inhibitors to modulate these transporters in intact tissues. For this effort, adult male C57BL/6 mice were injected intraperitoneally with HDAC inhibitors (valproic acid, VPA, or apicidin) once daily for seven days and different regions of the brains were collected for analysis of mRNA and protein expression of Mdr1 and Bcrp by qPCR and western blot, respectively. Treatment with HDAC inhibitors had no significant effects on transporter mRNA expression at 7 days. On the other hand, VPA increased the protein expression of Mdr1 in striatum by 70% and Bcrp in midbrain by 30%. Apicidin also enhanced striatal Mdr1 protein by 30% and hippocampal Bcrp protein expression by 20%. Transporter up-regulation in these brain regions was associated with increased histone H3 (H3/K14) acetylation. In summary, our findings indicate that HDAC inhibitors significantly up-regulated the expression of Mdr1 and Bcrp proteins in specific regions of mouse brains. Pharmacological inhibition of HDACs may alter transport properties in the brain and consequently modulate brain levels of neuroactive drugs and neurotoxins. Supported by a Predoctoral Fellowship from Bristol-Myers Squibb, P30ES050522, and R01ES021800.

Area under Curve and Maximum Concentration Exposures and Time-Kill Response Relationships In Vitro Hollow Fiber Infection Model System

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Antibiotics by means of humanized pharmacokinetic schedules in interaction with infecting bacteria have the potential to predict antibiotic-resistances. Antibiotics including ampicillin (45 μg/ml concentration), fosfomycin (450 μg/ml concentration) and ciprofloxacin (0.72μg/ml concentration) and their dual combinations were in vitro applied pharmacokinetically against a clinical isolate, uropathogenic Escherichia coli strain CFT073 bacteria at 37°C. Approximately, 10^6 CFU/mL of bacteria in monolayers in plastic wells. Chemicals can distribute between the plastic, media, and the cells, but it is unknown for what fraction of the Tox21 chemical space and to what extent this differential partitioning between in vitro compartments occurs. These initial studies evaluate the in vitro distribution of chemicals over a period of 24 hours, the length of many of the Tox21/ToxCast experiments. This pilot study involves the use of MCF-7 cells exposed to a diverse set of ten chemicals. A Thermo Vanquish LC system is used in conjunction with a Thermo Q Exactive Plus mass spectrometer to determine the concentration of chemicals in the media, plastic, and cells. Results from this pilot show that at 24 hours the concentration of chemical in cells compared to the concentration in media can vary from 12-fold (atrazine) to 195-fold (triphenyl phosphate). This gives quantitative insight into the magnitude and directionality of the uncertainty in the assumption that media to cells and blood to tissue fosfomycin at AUCmax/MIC being 7.7 μg -hour/ml and C0-24/MIC being 1.6 μg/ml had inhibited the replication of resistant bacteria for first 5 to 7 hours. In dual combination experiments using same antibiotics, together they could inhibit the replication of resistance for 24 hours. The last doses in once daily experiments could significantly suppress bacteria to the range of 0 to 10^8 CFU/mL for the 48 hours and above. These findings suggest that, all antibiotics with respect to their exposure-response relationships could be relied to treat Escherichia coli of CFT073 causing uncomplicated urinary tract infection. From a pharmacodynamic viewpoint, these findings can be used to prescreen and minimize toxicities of antibiotics in treating infections.

Impact of Pegylation in Pharmacokinetic (PK) Assessment of Pegfilgrastim Using Alternate Assays

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Polyethylene glycol (PEG), a flexible, uncharged and highly hydrophilic polymer, is used to prolong the half-life and improve efficacy of therapeutic proteins and nanoparticles. Despite these key advantages of PEG conjugation, the biosimilars developed from their respective originator products may fail to meet requirements for approval. This could be broadly attributed to the presence of PEG, which can present challenges in the bioanalytical methods to assess serum concentrations of the PEGylated compound for PK studies. In addition, aggregate formation, steric hindrance, chemical properties of PEG (in size, length, conjugation density) and functionalization conditions could also attribute to the PK variability. As there are over a dozen PEGylated products currently marketed, there is an urgent need to understand the potential causes for variability in PK of PEGylated products. There has been intense development of pegfilgrastim biosimilar products, so we chose to use this product and its non-PEGylated counterpart as the initial products to evaluate. We evaluated three different ligand binding assays (LBA) that could be used by sponsors and followed US FDA’s Bioanalytical Method Validation guideline for initial validation. Parameters assessed included sample stability at different temperatures, effect of matrix, use of apigdard analyte, accuracy, recovery of recovery of mono- and di-PEG conjugates. Using the optimized and qualified assay methods, inter-conor variability in serum from 50 healthy donors were also evaluated for both pegfilgrastim and filgrastim. Two of three assays demonstrated performance within guideline recommendations for filgrastim and pegfilgrastim for both fresh and frozen samples. The third assay was not able to be validated for filgrastim, but not pegfilgrastim due to differences in both fresh and frozen sample results. The paired comparison between different LBA methods demonstrated key differences, which are critical for their applicability for PK assessment.

Where is the Chemical?: The In Vitro Disposition of Tox21 Chemicals

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Understanding the in vitro disposition of chemicals evaluated in alternative models would enable better translation of in vitro bioactivity to the human dose context. In an effort to extrapolate in vitro exposures to in vivo exposures, toxicokinetic models can be used to estimate human oral exposures that would result in blood concentrations equivalent to the nominal media concentrations. One assumption in these models is that the ratio of the chemical concentration in media to cells is equivalent to the ratio of blood to tissue. The other assumption is the static nature of the cell culture and exposure compared to the kinetic processes occurring in vivo. In contrast, human exposure is a dynamic process in which absorption, distribution, metabolism and elimination influence blood and tissue chemical concentrations. In the Tox21 project most of the assays employ immortalized cell lines cultured in monolayers in plastic wells. Chemicals can distribute between the plastic, media, and the cells, but it is unknown for what fraction of the Tox21 chemical space and to what extent this differential partitioning between in vitro compartments occurs. These initial studies evaluate the in vitro distribution of chemicals over a period of 24 hours, the length of many of the Tox21/ToxCast assays. This pilot study involves the use of MCF-7 cells exposed to a diverse set of ten chemicals. A Thermo Vanquish LC system is used in conjunction with a Thermo Q Exactive Plus mass spectrometer to determine the concentration of chemicals in the media, plastic, and cells. Results from this pilot show that at 24 hours the concentration of chemical in cells compared to the concentration in media can vary from 12-fold (atrazine) to 195-fold (triphenyl phosphate). This gives quantitative insight into the magnitude and directionality of the uncertainty in the assumption that media to cells and blood to tissue
Toxicokinetics can be used to extrapolate an oral equivalent dose from in vitro bioactivity data for comparison with potential external exposure rates, thereby providing an estimate of risk. More accurately predict the oral equivalent dose, it is desirable to estimate the oral bioavailability (F_int), which is the fraction of the oral dose that is actually available to the body. Caco-2 assays inform estimates for F_int by providing a measure of the in vitro apparent permeability (P_app \text{ cm/s}) across a membrane of human colon carcinoma cells. This permeability is highly correlated with the fraction of chemical absorbed (F_int) in the gut and the effective permeability rate (P_eff \text{ cm/s}) through the epithelium of the small intestine (Artursson, et al. 2001). Predicted P_eff may then be used with in vitro measured intrinsic hepatic clearance (Cl_int) to estimate the fraction of chemical surviving gut metabolism (F_g) (Yang, et al. 2007). Subsequently, F_bio can then be determined by combining F_g and F_int with the fraction of chemical surviving first pass hepatic clearance, with the result being Cl_int and in plasma. In this work, we developed a random forest QSAR model to predict P_app using recently measured values for environmental chemicals and literature values for pharmaceuticals. We then used the predicted P_app to estimate P_eff \text{ cm/s} and F_bio for comparison to literature values. Using a training set of 166 chemicals, the QSAR model provided reasonable prediction of the P_app for the validation dataset of 315 chemicals, yielding an RMSE of 0.60 for the log_{10} transformed values. Using the QSAR predicted P_app to predict P_eff gave an RMSE of 0.69 for the log_{10} transformed values. Predictions for F_g in humans made using the model reported by Darvich et al. (2010) had an RMSE of 0.26. Estimates for F_g in humans using the Q_m model (Yang et al. 2007) gave an RMSE of 0.26. Subsequent estimates of F_bio for human and rat had RMSE's of 0.32 and 0.47 respectively. With additional open-source models to predict Cl_int and F_g, it would be possible to make predictions for F_bio entirely using in silico methods. This abstract does not necessarily reflect US EPA policy. Artursson, et al. Adv. Drug Deliv. Rev. 2001, 46, 27-43. Yang, et al. Current Drug Metab. 2007, 8, 676-684.

### QSAR Modeling of Caco-2 Permeability for the Estimation of Oral Bioavailability

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Understanding saturation of absorption and elimination of parent chemical and biomarkers/metabolites in repeated dose toxicity studies provides insight into treated level selection for subsequent toxicity studies based on the kinetically-derived maximum dose (KMD). Knowledge of toxicokinetics (TK) and non-linearity of blood/urine concentrations of parent and metabolites aids in study interpretation and the relation between external exposure and internal systemic dose. Experience with integration of TK into toxicity studies (without use of extra animals) has provided information on the usefulness of TK for dose level setting, internal dose determination and study interpretation. A TK decision tree/framework was developed based on observations with agrochemicals over ~15 years of TK assessments with respect to variation in absorption, metabolism and elimination. Examples of chemicals that have simple and complicated metabolism pathways, low and high absorption, saturation of elimination pathways, enterohepatic recirculation and a perspective of when KMD has been successful or not for dose level setting and retrospective study interpretation will be presented. Examples include chemicals (poorly and extensively absorbed and metabolized) where biomarker/metabolites include parent (sulfoxaflor), parent and 1 metabolite (halofenoxymethyl and acid, flonyrauxfen-benzyl), parent and 1-2 metabolites (fenpicloram), and other chemical examples of parent with greater than 2 metabolites. The developed decision tree includes design of studies to select biomarkers of exposure, selection of biomarkers based on absorbed dose, timing of TK analysis relative to subsequent studies, interpretation of biomarker concentrations relative to routes of exposure and study endpoints and the use of TK for metabolism studies to investigate differences across species. In case studies presented, it is possible to analyze for blood concentrations of parent/metabolites and determine whether dose-proportionality is observed in repeated dose toxicity studies and use the KMD for dose level selection, study interpretation and internal dose measurement. There is high value in understanding the TK of a chemical and its metabolites in mammalian toxicology studies, the internal systemic exposure to the animal and the extrapolation of the internal dose to human exposure assessments.

### Integration of Toxicokinetics into Toxicity Studies: TK Decision Tree/Framework for Agrochemicals

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### An Open-Source, Generalized Workflow for IVIVE Analysis


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4. NIEHS/NICEATM, Research Triangle Park, NC.

A critical challenge for implementing nonanimal approaches for chemical safety testing is to translate in vitro assay results to potential in vivo effects. To address this challenge, we have developed an open-source in vitro to in vivo extrapolation (IVIVE) workflow. The workflow incorporates in vitro data, quantitative structure-property relationships (QSPR) predictions, and one-compartment pharmacokinetic (PK) or multi-compartment physiologically based PK (PBPK) models to predict in vivo exposures that would result in blood concentrations equivalent to in vitro activity concentrations. We previously applied the IVIVE-PK workflow to evaluate estrogenic activity for environmental chemicals and observed good concordance between in vitro and in vivo dosimetry. However, for chemicals with poor oral bioavailability (e.g., bisphenol A, 17β-estradiol), the IVIVE-PK workflow tended to underestimate in vivo exposure levels, possibly due to a lack of extrahepatic metabolism in the one-compartment PK model. In this study, we developed a multi-compartment PBPK model that includes gastrointestinal glucuronidation to simulate a chemical’s plasma and tissue concentration after oral administration. We incorporated this PBPK model into the IVIVE workflow (IVIVE-PBPK) to estimate equivalent administered in vivo dose levels based on in vitro estrogenic activity concentrations for bisphenol A and 17β-estradiol, both of which are known to undergo glucuronidation, and achieved improved results. To apply this IVIVE-PBPK workflow to a larger chemical space, we also developed in silico models to predict kinetic constants for glucuronidation and other pharmacokinetic parameters. The IVIVE-PBPK workflow will be available via the NICEATM Integrated Chemical Environment (ICE) at https://ice.ntp.niehs.nih.gov, and a wide range of in vitro data within ICE, including CurtoX data, are available for workflow input. This optimized approach for using in vitro data to quantitatively predict in vivo effects is presented via an online tool that is accessible to a diverse stakeholder community and is designed to increase the utility of in vitro data in risk assessment applications. This project was funded with US federal funds from the NIEHS/NIH/HHS under Contract HHSN272201500010C.

### Ca2+-Dependent Gene Expression in Response to PCB95 and Triclosan Exposure

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Health issues including neurodegenerative diseases and cardiac and skeletal muscle myopathies are strongly associated with cellular calcium signaling disruption (CSD). Two channels that contribute to Ca2+ signaling are the ryanodine receptor (RyR), a Ca2+ release channel imbedded in the sarco/endoplasmic reticulum, and L-type voltage-gated calcium channels (CaVι), on the plasma membrane that regulate Ca2+ entry upon cellular depolarization. Environmental pollutants such as non-coplanar polychlorinated biphenyls (nCPCBs) and triclosan (TCS) can alter RyR and CaVι function and may be related to altered cellular signaling. The project investigates whether CSD, caused by the nCPCB, PCB95, and TCS, may lead to altered transcription of genes regulated by the Ca2+-dependent transcriptional regulators DREAM (downstream regulator of the CaVι) and Distal C-terminal (dCT) of the CaVι channel. Using the GHR somatotrophic cell line, I measured time and concentration dependent impacts of chemical exposure on the expression of DREAM-regulated prolactin (PRL), neuronal PAS domain protein 4 (NPAS4), and brain-derived neurotrophic factor (BDNF), as well as dCT-regulated sodium-calcium exchanger (NCKΙ) and connexin (CΧ31.1). Here, cells were exposed to 0.1μM, 1μM, and 10μM concentrations of PCB95 or TCS for 3-, 6-, 12-, and 24-hr with subsequent qPCR. Preliminary results indicate upregulation of gene expression for CΧ31.1 after 6-hr exposure to 10μM TCS and NPAS4 after 3-, 6-, 12-, and 24-hr exposure to 10μM TCS. This work will help extend our knowledge of pollutant induced CSD by non-coplanar compounds and to determine whether CSD can contribute to altered transcriptional regulation with implications in endocrine, cognitive and striated muscle health in exposed organisms.
3141 Calcium Signaling Disruption Causes Changes in Gene Transcription as Mediated by DREAM

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Several environmental pollutants, including polychlorinated biphenyl (PCB) congeners and triclosan, are capable of causing Ca2+ signal disruption (CSD) by altering the activity of the ryanodine receptor (RyR) or the L-type voltage-gated Ca2+ channel (CaV1). These channels are important to neuronal and muscular physiology, but the extent to which CSD through these channels contributes to altered cellular pathways is currently unclear. We investigated whether CSD, caused by cellular exposure to PCB congeners and triclosan, alters transcription normally regulated by the Ca2+-sensitive transcriptional repressors DREAM (downstream regulatory element antagonistic modulator). When intracellular Ca2+ concentrations are decreased, DREAM remains bound to DNA and represses transcription, and at high intracellular Ca2+ concentrations, DREAM is released from DNA and activates transcription, representing a direct connection between CSD and transcription. We utilized GT1-7 hypothalamic neurons and ToT1 thyrotrrophs to measure whether CSD alters transcription of gonadotropin releasing hormone (GnRH), thyroid stimulating hormone (TSH), neuronal PAS domain protein 4 (NPAS4) and brain-derived neurotrophic factor (BDNF). Cells were exposed to varying concentrations of each pollutant for multiple time periods and GnRH, TSH, NPAS4, and BDNF levels assessed using qPCR. GT1-7 cells exposed to the potent RyR activator CB952 did not lead to changes in GnRH mRNA expression consistent with low basal RyR gene expression. Exposure of GT1-7 cells to triclosan, a CaV1 inhibitor, significantly decreased GnRH transcription in a dose and time dependent manner. Exposure of ToT1 cells to CB952 showed no significant changes in TSH, and beginning analysis of NPAS4 and BDNF transcription are trending upward. DREAM is important to proper functionality of the digestive system, central nervous system, and skeletal and cardiac muscle, and it has been tied to pain response and memory, and thyroid-gland health. This work will help address whether CSD is contributing to such alterations by altering DREAM-mediated transcription.

3142 Assessment of Avobenzene, Ensulizole, Homosalate, and Padimate-O in Endocrine Disruptor Screening Panel Studies

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Avobenzene (ABZ), Ensulizole (ESZ), homosalate (HMS) and padimate-O (PMO) are UV absorbing agents commonly used in sunscreens. The potential estrogen and androgen effects of these compounds were evaluated in vitro; estrogen receptor (ER) and androgen receptor (AR) binding and transcriptional activation and in vivo; rat uterotrophic and Hershberger bioassays, respectively. ABZ, ESZ, HMS, and PMO did not increase ERα-mediated luciferase activity in the HeLa-9903 system at any of the viable concentrations tested up to the limit of solubility. All four were classified as a “non-binders”. ABZ, ESZ, HMS did not demonstrate AR agonism, however there was an apparent estrogenic activity in the Hershberger assay. In the rat Hershberger bioassay, each pollutant for multiple time periods and GnRH, TSH, NPAS4, and BDNF levels assessed using qPCR. GT1-7 cells exposed to the potent RyR activator CB952 did not lead to changes in GnRH mRNA expression consistent with low basal RyR gene expression. Exposure of GT1-7 cells to triclosan, a CaV1 inhibitor, significantly decreased GnRH transcription in a dose and time dependent manner. Exposure of ToT1 cells to CB952 showed no significant changes in TSH, and beginning analysis of NPAS4 and BDNF transcription are trending upward. DREAM is important to proper functionality of the digestive system, central nervous system, and skeletal and cardiac muscle, and it has been tied to pain response and memory, and thyroid-gland health. This work will help address whether CSD is contributing to such alterations by altering DREAM-mediated transcription.

3143 Toxic Effects of UV Filter Chemicals (Benzophenones and Octocrylene) on Zebrafish Embryo-Larvae

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Ultraviolet (UV) filter chemicals found in sunscreen materials are common environmental contaminants that represent a growing health risk concern due to their presence in water, fish, and human systems. Benzoresorcinol (B1), oxazobenzene (B3), dihydrobenzoxazole (B8), and octocrylene (OC, CAS 6197-30-4) are four such chemicals that have been detected in environmental samples and linked to alterations in estrogen receptor signaling pathway and producing oxidative stress. Zebrafish is a well-established model to study molecular genetics, toxicology, and trans-generation effects of chemicals. We have previously used an in vitro model of ZFL liver cell-line to test the toxicities of these chemicals. In this study, zebrafish (AR strain) embryo-larvae was employed to investigate the potential risks of the benzophenones (BPs) and OC, and the molecular mechanism of their toxic effects. Chemical exposures (24-hour and 96-hour) were conducted on larvae to determine half lethal concentrations (LC50s) for each chemical and hatching rate on embryo at each 24 hours. Gene expression profiles on estrogen receptor (ER) pathway, aryl hydrocarbon receptor (AhR) pathway and gender differentiation have been detected by using quantitative real-time PCR (qRT-PCR). Preliminary data showed that OC has no obvious effect on the hatching rate of embryos, and did not cause larvae death even at high concentrations. However, it was found that OC strongly up-regulated the expression of ERs and other sex-determining genes by gene profiling using qRT-PCR. Based on this result, it was speculated that OC will cause serious interference to endocrine systems in vertebrates, and we are studying the chemical ingestion in the exposed embryo-larvae. On the contrary, BPs reduced the hatching rate of the embryos and caused larvae deformity with heart swelling. More importantly, the LC50 values of BPs on larvae is comparable to the concentrations detected in European countries. From our qRT-PCR data, all three BPs induced gene expression level of both ER pathway and AhR pathway. Research supported by a Direct Grant from Faculty of Science, Chinese University of Hong Kong.

3144 CLARITY-BPA Core Study: Analysis for Non-monotonic Dose-Responses

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A recently published study sponsored by the European Food Safety Authority (EFSA) described a methodology for evaluating non-monotonic dose responses (NMDR) by assessing study findings according to 6 checkpoints. The publication (Varret et al., 2018) suggests researchers consider a meta-analysis of available data when a finding fulfilled at least 5 of the 6 checkpoints. This methodology was applied to the recently released results of a large U.S. government-sponsored 2-year bisphenol A (BPA) rat study. The BPA study was a collaborative effort between the US FDA (FDA), the National Toxicology Program (NTP), and the National Institute for Environmental Health Sciences (NIUHS) and is called the Consortium Linking Academic and Regulatory Insights on Bisphenol A Toxicity (CLARITY-BPA) Core Study. It involved oral gavage treatment of rats to BPA at doses of 2.5, 25, 250, and 25,000 ug/kg/day and was designed to address some of the lingering toxicological issues associated with BPA, including its possible role in endocrine disruption and the potential to induce NMDR. The study was conducted according to guideline research standards and included two arms: in utero through perinatal exposure (stop-dose arm) and in utero through lifetime exposure (continuous-dose arm). The evaluation presented herein provides additional analyses of the data, beyond those of the study report, that focus on the statistically significant findings. Only 2 of the statistically significant findings met at least 5 of the 6 checkpoint requirements for NMDR according the EFSA-sponsored publication; these were for clinical chemistry changes in serum: an increase in percent basophils in stop-dose females and decreased total bile acids in stop-dose males. However, further evaluation showed these 2 findings to not be biologically significant. Overall, this analysis found little evidence for NMDR or biologically significant changes associated with BPA treatment in the CLARITY-BPA Core study.
3145 Influence of Bisphenol A on hCG Release, Differentiation and Proliferation of Human Trophoblast Cells
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BPA is an endocrine disrupter and is one of the most utilized industrial chemicals worldwide. Epidemiological and animal studies provide evidence that environmental BPA exposure can be harmful to humans. Several studies showed that exposure to low doses of BPA, particularly during prenatal development and early infancy, BPA can pass through the placenta and reach the fetus, as revealed by the concentrations in amniotic fluid, cord blood and placental and fetal tissues. BPA exposure to the developing fetus is of particular concern as the compound readily crosses the placental barrier and accumulates both in the placenta and in the fetus. Hormones, such as E2, progesterone and hCG are fundamental in pregnancy. Moreover, hCG has endocrine, paracrine and autocrine actions on a variety of cells and tissues. These actions are directed to promote trophoblast invasiveness and differentiation, placental growth, hormone production and fetal growth. Therefore, any interference with their action might be harmful in pregnancy and for fetal health. This study was aimed to examine the effect of BPA on estrogen and progesterone-induced cell proliferation and the release of hCG in the human placental trophoblast (BeWo) cells. Cells were treated with BPA, estrogen or progesterone at various concentrations alone or in combination. Cell proliferation, cell death and level of hCG were evaluated. Exposure to BPA for 24h significantly decreased cell viability in a concentration-dependent manner. BPA at low concentrations (0.01nM-10nM) significantly potentiates estrogen or progesterone-induced cell proliferation. The estrogen antagonist, ICI 182,780, and progesterone antagonist, mifepristone, blocked these effects, indicating the effect of BPA on steroid receptors involved in the control of cell proliferation and differentiation in the placenta. At low concentrations of BPA, hCG release was significantly reduced at 24h. The decreased hCG release indicates reduced trophoblast function. Our data suggest that maternal contamination with BPA could disrupt placental endocrine activity which, in turn, could lead to adverse effects on pregnancy outcome, the fetal development and possibly long-term effects in the adult life. Supported by Title III.

3146 In Vitro and In Vivo Evidence Suggest Estrigenic Potential of Cyanobacterial Microcystin-LR via Stimulating Steroidogenesis
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Microcystins (MCs), produced by toxic cyanobacterial blooms that appeared world widely in eutrophic waters have the potential for disrupting endocrine homeostasis in aquatic animals. Thus far, the claim that MCs act as xenoeithogens remains uncertain. Using a combination of in vitro and in vivo assays, the present study investigated the endocrine disruption and estrogenic potential of the most toxic and common variant, microcystin-LR. Effects of microcystins (10, 100, 1000, 10000 μg/L) on steroidogenesis were assessed in in vitro H295R cells after 48 h. The contents of 17β-estradiol (E2) and testosterone (T) in the medium increased in a non-dose-dependent manner, which showed positive correlations with the expression of steroidogenic genes in H295R cells. In the in vivo assay, histopathological changes and hormone levels in the testis, gene expressions along the hypothalamic-pituitary-gonadal axis (HPG), especially the gonadal aromatase gene (cyp19a), were assessed. Meanwhile, spermatid percentage in the testis declined. In the liver, vitellogenin (vtg1) gene was significantly up-regulated while both the transcriptional and protein levels of ERα declined dramatically. The present results indicate that MC-LR induced non-dose-dependent estrogenic effects at environmental concentrations, which may result from steroidogenesis stimulation via a non-ER-mediated pathway. Our findings support a paradigm shift in the risk assessment of microcystin-LR from traditional toxicity to estrogenic risk, particularly at low concentrations, and emphasize the tremendous threat to the reproductive capacity of wildlife and even human beings in bloom areas.

3147 Estrogen Receptor Alpha-Coregulator Interactions and Transcriptomic Analysis of T47D Slightly Discriminate Diethylstilbestrol from 17β-Estradiol

Diethylstilbestrol (DES) is a synthetic estrogen and is a proven human teratogen and carcinogen. It has been reported that a functional estrogen receptor (α) is needed for DES-induced adverse effects. Since other estrogens that also activate the ERs, like 17β-estradiol (E2), do not show the adverse effects induced by DES in vivo, we hypothesized that DES’ interaction with the ERs differs from that of E2. Therefore, the current study aimed to investigate dose-specific differences in DES and E2 using in vitro assays related to ERα-mediated effects. To this end, the effects of DES and E2 on ERα-mediated reporter gene expression, ERα-mediated breast cancer cell (T47D cell) proliferation, ERα-coregulator interactions and transcriptomic analysis of T47D cells were studied. Results obtained indicate that DES activates ERα-mediated reporter gene transcription and T47D cell proliferation similar concentrations as E2. Furthermore, only minor differences between DES and E2 induced binding of the ERα to coregulator peptides and transcriptomic signatures obtained in the T47D breast cancer cell line were found. Although some of the minor differences observed may play a role in the differential in vivo effects of DES and E2, other modes of actions including possibly epigenetic mechanisms and/or differences in kinetics, may provide better explanations for the differences in in vivo effects of DES and E2.

3148 Towards Computational Modeling of Estrogen Receptor Alpha-Mediated Signaling in Endocrine Disruption
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Estrogen receptor alpha (ERα) belongs to the nuclear hormone receptor family of ligand-inducible transcription factors, and regulates gene networks in biological processes such as cell growth and proliferation. Disruption of these networks, for instance in breast cancer cells in the presence of the non-endocrine carcinogenic 17β-estradiol (E2), can result in adverse outcomes such as unanticipated cell proliferation ultimately culminating in tumor formation. Since ER signaling is also involved in normal physiological responses, and not solely activated in adverse outcomes, it is essential to define quantitative relationships between different key events leading to a particular adverse outcome induced by non-physiological ER activation. To obtain quantitative information on these key events, a technique is favored which can provide single cell information on all these events. For this purpose, we established fluorescent protein reporter cell lines with bacterial artificial chromosome (BAC) green fluorescent protein (GFP) transgenomics of important players in the ERα signaling pathway. The human breast cancer cell line MCF7 was used as a model as its proliferation is dependent on ERα. In order to determine relevant target genes of ERα, we used transcriptional data of MCF7 cells exposed to either E2 or a combination of E2 and 4-hydroxytamoxifen, an ERα-antagonist. The candidate genes were validated by qPCR to determine the E2-dependence; siRNA knockdown of ESR1 showed ERα-dependent induction of these genes. Finally, siRNA knockdown of these target genes led to a reduced proliferation rate, identifying these genes as relevant ERα targets genes in the context of proliferation. Our fluorescent protein reporters enable to monitor the following key events: receptor activation, downstream transcriptional activation and cell cycle progression, driving ultimate enhanced overall proliferation. In combination with advanced live cell imaging, these reporters can monitor the spatial and temporal dynamics of these key events at a single cell level. This adverse outcome pathway-driven reporter platform will allow us to determine the quantitative relationships between the different key events and the precise cellular adverse outcome. These in vitro reporters can be used to screen e.g. drug candidates or other chemicals of concern for the potential of modulating ER activity and likely non-genotoxic mode of action.

3149 Development of a Computational Model to Identify Potential Aromatase Inhibitors

In the final step of estrogen biosynthesis, the cytochrome P-450 enzyme aromatase (CYP19A1) converts testosterone to estradiol. Inhibition of aromatase is one of the primary screening targets for potential endocrine disruption.
The goal of our work was to mine existing public data and develop an in silico model that predicts the likelihood that an unknown compound will or will not inhibit aromatase. We compiled a database of substances comprised of >2000 aromatase inhibitors and >1300 non-inhibitors from multiple sources (e.g., ToxCast, Tox21, published validation data) and using various aromatase assays. We developed a random-forest machine-learning prediction model that determines potential inhibitory relationships between 15526 compounds, 10 E2, and 10 vtg1 RNAs to identify steroid hormones, isoflavones and estrogens, isoflavones and estrogens, and protein-docking binding energies. Our prediction model was built by iteratively and randomly retraining it on 90% of the known active and inactive compounds. Our model showed very high sensitivity (96%) and balanced accuracy (94.2%), with a specificity of 92.4%. The positive and negative predictive values were 96.2% and 92.2%, respectively. The model was then applied to ~2000 aromatase inhibitors falling into several scaffold classes (e.g., steroids, isoflavones and azoles) and showed a distinct trend relative to controls for the predicted binding energies.

**3150 Quantitation of Steroid Hormones for Endocrine Disruptor Screening Using a Multiplex Immunoassay**


The US EPA offers guidelines for testing substances that have the potential to interact with the hormone system. This is referred to as the Endocrine Disruptor Screening Program (EDSP) and is intended to determine if pesticides create health or environmental risk humans or ecological systems. The steroidogenesis assay requires H295R cells, a steroid producing adrenocortical cell line, to identify adverse effects on the steroidogenic pathway by measuring production of testosterone and estradiol. Test chemicals forskolin and prochloraz, a model inducer and inhibitor, respectively, were added to the H295R cells at increasing concentrations. The US EPA EDSP guidelines were followed to plate H295R cells and expose the cell line to test chemicals. Induction with forskolin and inhibition with prochloraz were performed along with DMSO solvent controls. Cell supernatant were collected after 48 hours stimulation. The Milliplex Multi-Species Hormone Magnetic Bead Panel was used to measure the chemical influence on H295R cells and verified according to the US EPA criteria. Testosterone met the US EPA criteria for induction by demonstrating ≥2x concentration with 10 µM forskolin (4,863 pg/mL) compared to the solvent control (SC) (2,432 pg/mL). Testosterone also met the criteria for inhibition with the concentration decreasing ≤0.5x the SC (2,438 pg/mL SC vs. 73.2 pg/mL with 1 µM prochloroz). Estradiol levels following induction also met US EPA criteria increasing ≥7.5x SC (106.7 pg/mL SC vs. 1066.8 pg/mL with 10 µM forskolin). The criteria were also achieved with the addition of the inhibitor, reading ≤0.5x SC (102.2 pg/mL SC vs. 7.8 pg/mL with 1µM prochloroz). In addition, the data generated using the Milliplex Multi-Species Hormone Magnetic Bead Panel was evaluated by mass spectrometry. Measurement of testosterone levels by LC-MS/MS was based on a previously described workflow utilizing Currillant Testosterone Certified Reference Materials (CRMs). The data generated from our study demonstrates the utility of endocrine disruptor testing using the Milliplex MAP Multi-Species Hormone Magnetic Bead Panel.

**3151 In Vivo High-Throughput Screening System for Estrogenic Endocrine Disruptors Using a Fluorescently-Labeled Zebrafish Line**

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Evidence of exposure to estrogenic compounds has substantially increased over the past decade affecting reproductive health in humans, domestic animals and wildlife. There is a need for development of time- and cost-effect screening tools for biological reporting of endocrine disruptors in food and the environment, as well as for screening new compounds for estrogenic activity. This is referred to as the Endocrine Disruptor Screening Program (EDSP) and is intended to determine if pesticides create health or environmental risk humans or ecological systems. The steroidogenesis assay requires H295R cells, a steroid producing adrenocortical cell line, to identify adverse effects on the steroidogenic pathway by measuring production of testosterone and estradiol. Test chemicals forskolin and prochloraz, a model inducer and inhibitor, respectively, were added to the H295R cells at increasing concentrations. The US EPA EDSP guidelines were followed to plate H295R cells and expose the cell line to test chemicals. Induction with forskolin and inhibition with prochloraz were performed along with DMSO solvent controls. Cell supernatant were collected after 48 hours stimulation. The Milliplex Multi-Species Hormone Magnetic Bead Panel was used to measure the chemical influence on H295R cells and verified according to the US EPA criteria. Testosterone met the US EPA criteria for induction by demonstrating ≥2x concentration with 10 µM forskolin (4,863 pg/mL) compared to the solvent control (SC) (2,432 pg/mL). Testosterone also met the criteria for inhibition with the concentration decreasing ≤0.5x the SC (2,438 pg/mL SC vs. 73.2 pg/mL with 1 µM prochloroz). Estradiol levels following induction also met US EPA criteria increasing ≥7.5x SC (106.7 pg/mL SC vs. 1066.8 pg/mL with 10 µM forskolin). The criteria were also achieved with the addition of the inhibitor, reading ≤0.5x SC (102.2 pg/mL SC vs. 7.8 pg/mL with 1µM prochloroz). In addition, the data generated using the Milliplex Multi-Species Hormone Magnetic Bead Panel was evaluated by mass spectrometry. Measurement of testosterone levels by LC-MS/MS was based on a previously described workflow utilizing Currillant Testosterone Certified Reference Materials (CRMs). The data generated from our study demonstrates the utility of endocrine disruptor testing using the Milliplex MAP Multi-Species Hormone Magnetic Bead Panel.

**3152 Targeted Expression Profiling Identifies Potential ER Independent Mechanisms of Mammary Toxicants**

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Over 200 chemicals have been associated with altered mammary gland development and breast cancer, yet the molecular mechanisms by which this diverse set of chemicals exert adverse effects on the mammary gland remain poorly understood. In this study we investigated gene expression profiles of 8 suspected mammary toxicants using the TempO-Seq platform and a targeted gene expression panel, Breast Carcinogen Screen (BCScreen). The BCscreen panel consists of 500 genes representing biological processes relevant to breast cancer and mammary gland development. We compared gene expression profiles from MCF-7 cells treated with four concentrations of PFOA, PFHXA, PFNA, genistein, genistein, 1,4 benzoquinone (BQ), BPA, butyl benzyl phthalate (BBP), or tamoxifen for 24hrs. The known ER agonists genistein, BPA, and BBP had highly similar gene expression profiles and were enriched for genes associated with cell cycle and DNA repair, particularly at high doses; and these profiles were also similar to the profile for cells treated with 10 nM estradiol. These weak estrogens activated a subset of cell cycle genes regulated by AhR and ERα1 transcription factors. In contrast, PFOA, tamoxifen, and BQ had higher gene expression changes at the lowest dose (1nM) compared with higher doses (10 nM to 10 micromolar) and were enriched for genes associated with cell cycle. PFOA and tamoxifen expression profiles were similarly enriched for several regulatory motifs including ERα1, E2F1 and E2F4, identifying a subset of cell cycle genes suppressed in response to these toxicants. PFOA has no known direct ER activity, yet we found these expression profiles were highly correlated with gene expression profiles from several known breast ER antagonists, suggesting indirect suppression of ER-regulated cell cycle genes that may modulate effects on mammary gland development and breast cancer risk. Genes upregulated by PFOA, BBP, and genistein were enriched among genes differentially expressed in breast cancer, whereas genes suppressed by tamoxifen, PFOA, and BQ were enriched in breast cancer. These findings highlight the potential for low doses of mammary toxicants to alter cell cycle pathways via ER-independent mechanisms that affect mammary gland development and breast cancer susceptibility.

**3153 Sex-Specific Associations of Prenatal Exposure to Persistent Organic Pollutants with Placental DNA Methylation of Thyroid Hormone Related Genes among Koreans**

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Exposure to persistent organic pollutants (POPs) during pregnancy is associated with disruption in thyroid hormone balance. The placenta not only regulates thyroid hormone supply to the fetus but is also a key tissue in understanding the mechanisms for the developmental origins of disease. This study was conducted to evaluate the relationship between maternal serum and placental DNA methylation of thyroid hormone related genes. We measured the promoter methylation of several genes in placental samples collected from 106 Korean mother-child pairs by bisulfite pyrosequencing.
The thyroid-stimulating hormone receptor (TSHR) is a G protein-coupled receptor that signals through adenylate cyclase to increase intracellular 3',5'-cyclic adenosine monophosphate (cAMP) and activate cAMP-dependent protein kinase, resulting in increased thyroid hormone (TH) production in thyroid follicular cells. Due to human health effects resulting from altered TH levels, it is important to evaluate whether environmental chemicals can disrupt thyroid function via TSHR-mediated signaling pathways. As part of the Tox21 collaboration, HER293-TSHR cells were used in a 1536-well assay format to demonstrate agonism or antagonism of the TSHR, using cAMP as a marker of TSHR activation. Homogeneous time-resolved fluorescence technology was used to quantify cAMP using a competitive immunoassay between native and dye-labeled cAMP. Out of the 7,872 tested chemicals, 6% agonist, 4% antagonist, and 0.6% agonist-antagonist hits were identified, for a total of ~10% putative active chemicals. Because receptor binding is highly specific, we hypothesized that many of the hits were false positives. Thus, we developed a novel prioritization scheme to select chemicals for screening in biologically-relevant follow-up assays. Chemicals (558/778 active chemicals) were clustered by structural similarity using ChemotypeToxPrint fingerprints. The priority score (within cluster and for non-clustered chemicals) was penalized for: i) activity in other ToxCast cAMP enzymatic assays, ii) promiscuity according to ToxCast total assay hit rate, iii) signal interference by autophosphorylation, and iv) cytotoxicity. Highly-ranked agonist clusters contain phenols, organochlorine insecticides, and retinoids. Cytotoxicity contributed significantly to the agonist priority rank, with as many as 68% of antagonists suspected to be cytotoxic in the active concentration ranges. The prioritization scheme has identified 69/778 [CJ1] [SA2] active chemicals that are structurally diverse for additional testing. Using this scheme, secondary screening of identified priority chemicals will be combined with structural prioritization to create an integrated predictive tool for TSHR activity. This abstract does not reflect US EPA policy.

**3157 Thyroid Disruption Screening Method Using Zebrafish Vertebrate Model**


Endocrine Disrupting compounds are being increasingly detected in the environment and may have a profound impact on the development and physiology of vertebrate organisms. Thyroid Disrupting (TD) compounds specifically alter the function of thyroid gland through the interference with the synthesis, transport and/or binding of the thyroid hormones. Several environmental contaminants such as polybrominated diphenyl ethers or halogenated organophosphates, used as plasticizers and flame retardants, are suspected to produce a TD effect. Given so, chemical manufacturing entities could benefit from cost-effective methodologies for the screening of TD substances in order to deselect candidates during the early phase of the development. The zebrafish vertebrate model is broadly used in both human and aquatic toxicity assessment due to their low cost, small size, rapid development, and homology with mammals. Besides, the existence of numerous transgenic strains enables to perform fluorescence-based screening assays with medium throughput. In this work, a screening method for TD substances assessment was developed using the Tg(tg:smcherry) transgenic zebrafish line. The fluorescence of the reporter gene allows monitoring in vivo the upregulation of the tyrosylbogulin gene expression as a compensatory reaction to thyroid gland disruption. In this assay, the exposure concentrations of test substances were selected according to the results of a preliminary acute toxicity assay to avoid any interference by non-specific toxicity. Afterwards, transgenic em...
bryos were exposed to the test substances from 48 to 120 hpf (hours post-fertilization) and subsequently imaged by fluorescence microscopy. A dose-dependent increase of the fluorescence was observed, and the intensity values were fitted to a concentration-response regression model to calculate TD predators, such as the Benchmark Concentration (BMC), Thyroid Disrupting Index (TDI), and Relative TD Potency (RTP). Finally, an rt-qPCR gene expression assay was developed over known markers of thyroid pathway (Tshβ, tgf, and tpo) to characterize the mechanism of action involved in the endocrine disrupting effect. The screening method presented in this work has been developed and validated using a set of 19 environmentally relevant TD substances. This screening methodology showed to be a sensitive and cost-effective assay to evaluate the potential thyroid disruptor activity of chemicals.

3158 Development of an In Vitro Human Thyroid Microtissue Model for Chemical Screening

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Thyroid hormones (TH) are essential for regulating a number of diverse physiological processes required for normal growth, development, and metabolism. The US EPA Endocrine Disruptor Screening Program (EDSP) has identified several molecular thyroid targets relevant to hormone synthesis dynamics that include the TSH receptor (TSHR), Sodium/Iodide Symporter (NIS), Thyroperoxidase (TPO), Dual Oxidase (DUOX), Iodotyrosine Deiodinase (IYD), and Pendrin. High-throughput screening assays for TSHR, TPO, and NIS have been used to screen chemicals across the Toxicast/Tox21 chemical inventories to identify potential thyroid disrupting chemicals (TDCs). The uncertainty surrounding the specificity of hits identified in these screens, the relevance to human biology, and the quantitative potency relationship of molecular perturbation to decreased TH hormone synthesis, have led to relatively large data gaps in hazard identification for TDCs. The objective of this study was to develop a medium-throughput organotypic screening assay comprised of reconstructed human thyroid microtissues to quantitatively evaluate the disruptive effects of chemicals on TH production and secretion. Cells procured from qualified euthyroid donors were analyzed for retention of NIS by well plate image analysis to verify enrichment of follicular epithelial cells. A direct comparison of two-dimensional (2D) and three-dimensional (3D) 96-well culture formats was employed to evaluate TH production and secretion over the course of 20 days. The results of serial sampling in the 2D format revealed considerable loss of TH expression with no subsequent production of TH3 or TH4. In contrast, the 3D model continued to increase TH levels over time, with sustained output of TH3 and TH4. Inhibition of TH synthesis in an optimized 3D culture format was demonstrated with reference chemicals Methimazole, 6-Propyl-2-thiouracil, and Sodium Perchlorate. Overall, the 3D thyroid microtissue assay will prove to be a valuable resource for extending screening efforts currently underway in Toxicast/Tox21 and EDSP. This abstract does not necessarily reflect the policy of the US EPA.

3159 In Vitro Approaches to Risk Assess Chemical Mediated Changes in Thyroid Function

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The hypothalamic-pituitary-thyroid axis (HPT axis) is conserved across vertebrate evolution. Perturbation of thyroid hormone homeostasis (THH) can lead to adverse effects in thyroid function affecting growth, metabolism and cognitive function. In vitro, appropriate thyroid hormone concentrations are absolutely required for normal nervous system development. Chemical disruption of THH can occur via a number of mechanisms, including increased hepatic thyroid hormone clearance, inhibition of iodide transport into the thyroid (sodium/iodide symporter), inhibition of iodide oxidation (thyroid peroxidase) and inhibition of thyroid hormone deiodination (deiodinases). To understand the effects of chemicals on these processes the following assays are utilised. 1. in vitro primary hepatic thyroid hormone metabolism (multiples species), 2. Thyroid peroxidase inhibition (multiple species), 3. Deiodinase inhibition (rat and human), 4. Sodium/iodide symporter inhibition (rat). Rat sodium iodide symporter inhibition and rat and human deiodinase 1,2 and 3 inhibition assays are currently being validated. Here we report the validation of in vitro rat, dog, pig and human TPO inhibition and in vitro primary hepatocyte metabolism of thyroxine (T4). In concurrence with the literature, TPO inhibition by 6-propyl-2-thiouracil (PTU) shows broad sensitivity across the species tested. In the dog (IC50 5.2 µM), the compound shows concentration-dependent inhibition of T4 metabolism in primary cultured dog hepatocytes showing a consistent dose response inhibition (approximately 2-fold over vehicle control) in response to item feature identification. These assays form part of an in vitro screen used to generate data to study chemical endocrine disruption hazard.

3160 Comparative In Vitro Investigation of Thyroxine Phase II Liver Enzyme Activities Using Rat and Human Hepatocytes to Address Species Differences in Thyroid Disruption

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The in vivo induction of hepatic phase II enzymes via activation of nuclear receptors has been well described in the literature for several mammalian species following exposure to xenobiotics. This induction of phase II enzymes is an early key event in the AOPs leading to increased metabolism of thyroid hormones and further potential thyroid and neurodevelopmental adverse effects. However, comparative species assessment of the level of induction of these phase II enzymes has not been thoroughly studied, even though the magnitude of this early change may play an important role in the observed species differences in liver mediated thyroid toxicity (e.g. rat vs dog). The activity of liver enzymes involved in thyroxine (T4) conjugation was thus, compared using cryopreserved hepatocytes from rats (Wistar) and human donors following 72h of cell culture in the presence or absence of various concentrations of reference liver enzyme inducers: phenobarbital (PB), pregnenolone 16a-carbonitrile (PCN), 6-naphthoflavone (BNF), UGT-T4 (phenol and acyl) and SULT-T4 activities were measured by LC/MS/MS following incubation with 100µM T4 for 4h (showing kinetic linearity). Results indicate that the basal level of SULT-T4 was similar between the two species and was not increased by the enzyme inducers. Basal levels of phenol UGT-T4 were at least 10 times higher in rat compared to human hepatocytes. PB did not induce UGT-T4 activity in either rat or human cells, PCN showed a maximum 2-fold increase in phenol UGT-T4 in rat hepatocytes but no increase was observed in human cells. BNF induced a concentration-related increase in phenol UGT-T4 (up to 5-fold) in rat hepatocytes and a marginal increase in human hepatocytes. The results indicate that T4 glucuron-conjugation capacity and the response to inducers is greater in rat hepatocytes compared to human hepatocytes. Overall this work highlights a need to standardize and validate these in vitro assays if they are to be used in a weight of evidence approach to address species differences in liver mediated thyroid toxicity.

3161 In Vitro Screening Assays for Chemical Inhibition of Mammalian and Amphibian Iodotyrosine Deiodinase

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Iodotyrosine deiodinase (IYD, dehalogenase) catalyzes the recycling of iodide from moniodotyrosine (MIT) and diiodotyrosine (DIT) and thus has an important role in retention of iodide for synthesis of thyroid hormone. In an effort to understand the susceptibility of IYD to chemical perturbation, 96-well plate in vitro assays were developed to screen chemicals for inhibition of mammalian and amphibian IYD enzyme activity. With recombinant human IYD enzyme and Xenopus laevis liver microsomal fractions, these assays were established using MIT as the substrate and 3-nitro-L-tyrosine (MNT) as the positive control, and tested against an initial set of ten chemicals (7 known/suspected IYD inhibitors and 3 non-inhibitors). There was strong cross-species agreement between human and Xenopus IYD in this initial test set of chemicals, with similar maximum inhibition and rank order potencies; however, a larger set of chemicals must be tested to complete this species comparison. Performance metrics support use of these assays for screening, with high Z’ factor (0.6 or greater) and low variability in the control chemicals (DMSO, NaOH, and MNT). The Toxicast phase 1, v2 chemical library was then screened with recombinant human IYD to identify chemicals that have the potential to disrupt the thyroid axis via this pathway. The majority of these chemicals did not inhibit IYD enzyme activity at a single, high concentration (target of 200 µM). Of the 293 unique Toxicast chemicals tested, 28 chemicals (9.6%) inhibited human IYD activity by 20% or greater, including 3 chemicals previously reported to inhibit IYD. Of these 28 chemicals, 7 produced inhibition of 50% or greater and were further tested in concentration-response mode to determine IC50s and rank order potency. These IYD inhibition assays can be used for future screening of large chemical libraries to expand the limited number of in vitro assays used to identify chemicals having potential to interfere with
thymus, thymic cortical lymphocytes, and thymic medullary lymphocytes. 

Toxicological effects of T-2 toxin include modifications to feeding behav-
ior, growth retardation, cardiovascular alterations, immunosuppression, and hemo-
stastic derangements. However, to date, effects on the central nervous system (CNS) have rarely been reported. In the present study, female Wistar rats were given a single dose of T-2 toxin at 2 mg/kg b.w. and were sacrificed at one, three, and seven days post-exposure. Histopathological analysis and transmission electron microscope (TEM) observations were used to investi-
gate injury to the brain and pituitary gland. Damage to the brain and pituitary 
at the molecular level was detected by real time-polymerase chain reaction 
(RT-PCR), western blot, and immunohistochemical assays. Liquid chromato-
graph-mass spectrometer/mass spectrometer (LC-MS/MS) was used to inves-
tigate T-2 toxin and metabolites in the brain. The results showed that perox-
ated phospholipid lesions were obvious in the brain at three days post-exposure; lesions in the pituitary were not observed until seven days post-exposure. Autothagy in the brain and apoptosis in the pituitary suggest that T-2 toxin may induce differ-
cent acute reactions in different tissues. Importantly, low concentrations of T-2 
toxin in the brain were observed in only one rat. Responsible for the above 
findings, T-2 toxin-induced acute lesions in the pituitary, skin, liver, and kidneys 
were divided randomly in three groups (n=6/each): Controls received corn 
oil; low dose DEHP group received 30 mg/kg/day DEHP (lowest observed ad-
verse effect level (LOAEL) for reproductive system) and high dose DEHP group 
received 60 mg/kg/day (LOAELx2) DEHP by oral gavage till the end of the 
adolescent period (37 days) starting at the 23rd day after birth. After eutha-
nasia, testis tissue homogenates were prepared in methanol:water mixture 
and centrifuged at 1500 rpm for 20 min at -8°C and supernatants were evap-
orated. The residues were derivatized and analyzed with GC-MS. The chro-
natograms were deconvoluted and aligned using AMDIS and SpectConnect 
software, respectively. Tests metabolic profiles of the study groups were 
compared by multivariate statistical techniques (PCA and PLS-DA). We found 
that metabolites "urea" and "erythrose 4-phosphate" were different among 
all study groups. Moreover, in high dose group, "urea" and "9,12,15-Z,Z,Z-
Z octadecatrienoic acid" levels were decreased and glucopyranose and panto-
thenic acid levels were increased vs. control. In low dose group, 1-hydroxy-
anthraquinone was increased and octadecanol, cholesterol and erythrose 
4-phosphate were decreased vs. control. The metabolic difference in high 
dose versus control was sharper than low dose group. When we compared 
low dose and high dose groups, L-alanine, hydroxyaspartic acid, octodeca-
enoic acid and L-norleucine levels were increased in high dose group. These 
findings show that DEHP may affect glucose, lipid and energy metabolism. 
It may also contribute the oxidative stress.

Perfluoroxyanoic acid (PFHxA) is a potential impurity and environmental 
degradation product of C6-based fluorotelomer products. Based on the con-
cern that perfluoroalkyl acids have endocrine activity, a hypothesis driven 
weight-of-evidence (WoE) analysis of PFHxA was conducted to evaluate endo-
crine disrupting properties as defined by World Health Organization (WHO). 
Following a comprehensive literature search within PubMed and Embase da-
tabase, five studies were selected: four were in vitro studies and one was a 
clinical study. A total of 21 in vitro and in vivo studies across species were identified for review. High Throughput Screening (HTS) assay data within the ToxCast/Tox21 da-
tabase were also included in this assessment. Studies identified consisted of 
Level 1 to 4 methods within the OECD Conceptual Framework for assessing 
endocrine disruptors (ED) were reviewed for reliability and endocrine end-
points extracted that represented lines of evidence for positive or negative 
activity across the estrogen (E), androgen (A), thyroid (T) and steroidogenesis 
(S) pathways. The endpoints extracted were ranked based on their specificity 
and sensitivity for identifying PFHxA activity across 8 endocrine hypotheses 
(e.g., E, A, or T agonist or antagonist or S inducer or inhibitor). Overall, PFHxA 
showed no endocrine effects in Japanese medaka, juvenile rainbow trout, 
chickens or reproductive parameters in northern bobwhite quail. In rodent 
studies, there was no significant activity associated with PFHxA exposure 
in endocrine endpoints identified in repeat-dose toxicity studies, a lifetime can-
cer study, or guideline and non-guideline reproductive and developmental 
studies. In vitro, there was weak or negative activity for T transport protein 
or activation of E, A, or T receptors. PFHxA was also negative for disrupting 
steroidogenesis in vivo and in vitro. Based on this WoE analysis across these 
disease pathways (e.g., E, A, T, and S), PFHxA exposure was found not to 
cause adverse effects associated with alterations in endocrine activity in any 
of the experimental models evaluated, as such would not be characterized as 
an ED according to the WHO definition.
Endocrine disrupting chemicals (EDC) are becoming increasingly prevalent in the environment and many are shown to accumulate within human tissues and interact with endogenous hormone receptors. One such EDC is organophosphate flame-retardants (OPFR). OPFR interact with multiple hormone receptors involved in homeostasis, including estrogen receptors (ER) and peroxisome proliferator activated receptor-γ (PPAR-γ). We have previously reported that adult exposure to a mixture of OPFR induces sex-dependent alterations of energy homeostasis and hypothalamic gene expression of anorectic and orexigenic neuropeptides on a standard chow diet. This dysregulation of energy homeostasis can cause an increase in susceptibility to metabolic disorders. In current studies, we repeated the previous adult sex exposure experiment with a common mixture of OPFR [tris(1,3-dichloro-2-propyl)phosphate, triphenyl phosphate, and tricresyl phosphate, 1 mg/kg/day each] for 4 weeks while adding a comparison between low-fat diet (LFD, 10% kcal fat) and a high-fat (HFD, 45% kcal fat) to generate a diet-induced obesity model. We recorded body weight, crude food intake, body composition, metabolic rate, locomotor activity, meal patterns, glucose and insulin tolerance, and plasma peptide hormone levels. Consistent with our previous results, OPFR exposure reduced body weight gain in LFD-fed males, but increased weight gain in HFD-fed males, while not altering female body weight. OPFR treatment also showed subtle sex-dependent effects on oxygen consumption, carbon dioxide production, energy expenditure, and activity. In males, OPFR exposure increased oxygen consumption and activity and reduced those parameters in females during the night (~0100-0400 h). Meal patterns in females fed a HFD were altered by OPFR treatment. OPFR treatment altered circulating levels of insulin and glucagon in females and ghrelin in males. No effect of OPFR exposure was found in glucose or insulin tolerance. We are currently processing hypothalamic samples to measure protein and gene expression of anorectic and orexigenic neuropeptides. In summary, our data indicates that adult OPFR exposure can induce, and perhaps exacerbate, the effects of diet-induced obesity in mice. Ingestions of plastic microbeads have been identified in different aquatic organisms globally. Potential acute toxicity of ingested microbeads could be characterized as physical effects including blockage of digestive system or biological effects linked to alterations in oogenesis process and detoxification processes. To identify the physical effects, behavioral changes and gene expression profiles of phase 1 detoxification enzyme gene (cytochrome P4501A, cyp1a) and oogenesis process related gene (vtg1) induced by plastic microbeads, different size ranges of polyethylene (PE) microbeads with colors (Cospheric Ltd, USA, polymer type verified by micro-Raman spectroscopy) were selected in this study because PE is the dominant microplastic found in Hong Kong coastal area and effluent samples. Adult zebrafish was used as model organisms because of its fully characterized genome and evolutionarily conserved behavioral responses. For the identification of upper and lower boundaries of microbeads ingestion, individual polyethylene microbeads with different sizes (10-22 µm, 45-53 µm, 90-106 µm, 212-250 µm and 500-600 µm) in 2 mg/L concentration were used for 96 hours exposure. For studying in behavioral changes or targeted gene expression profiles through real time PCR (qPCR), mixture of microbeads with different size (45-53 µm (blue), 90-106 µm (green) and 212-250 µm (white)) in effluent related concentration (11 particles/L), medium concentration (110 particles/L), high concentration (1,100 particles/L) were applied for 96 hours exposure. All concentrations used are relevant to the environmental concentrations found in local waters. Zebrafish behaviors were recorded by video and two observers (interrater reliability > 85%). Results implied that the upper boundary and lower size boundary for microbead ingestion were 500-600 µm and 10-22 µm respectively. In addition, 60 % and 30 % of fish ingested and retained with microbeads in intestine (medium and high concentration) and gills (high concentration). Microbeads could cause less void inside the intestine. The expression of cyp1a in intestine (medium concentration) and vtg1 in liver (medium and high concentration) were upregulated significantly in the zebrafish exposed to PE microbeads. Zebrafish with abnormal behaviors (i.e. seizure and tail bent downward) were observed (medium and high concentration). In summary, effects on aryl hydrocarbon receptor (AhR) pathway, disruption of oogenesis process and neurotoxicity in adult zebrafish could be caused by acute exposure to microbeads.
3170 Identification of microRNA Signatures following Multigenerational Exposure to Deoxynivalenol in Caenorhabditis elegans

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Deoxynivalenol (DON) is one of the most widely distributed trichothecene mycotoxins commonly found in cereal grains and its ubiquitous contamination has become a big concern in the field of public health and food safety due to its potent acute and chronic toxicities to animals and humans. We previously demonstrated that DON produced significant toxic effects on growth, brood size and feeding behavior following multigenerational exposure to Caenorhabditis (C) elegans model. Because it has been well known that DON is not a genotoxic agent, we postulate that DON exposure may induce changes in the epigenome that can be used as potential molecular signatures of DON-induced toxicity. In this study, the total RNA in the worm groups treated with 200 μg/mL DON and control vehicle at different generation time (P0, F1, F2 and F3) were prepared, and the RNA sequencing was performed on Illumina NextSeq 500. The differentially expressed miRNAs were evaluated between the two treatment groups at different generation times. Compared to the control group, totals of 161 miRNAs (83 up- and 79 down-regulated), 177 miRNAs (110 up- and 67 down-regulated), 217 miRNAs (143 up- and 74 down-regulated), and 225 miRNAs (105 up- and 120 down-regulated) were differentially expressed following DON exposure at P0, F1, F2, and F3 generations, respectively. Further functional enrichment and bioinformatics analyses showed that the major alterations of these differentially expressed miRNAs were related to the protein synthesis, transcription, sex determination, balance of redox status, activity of kinases, and biotransformation. These data strongly suggest that alterations in miRNAs expression could serve as sensitive signatures for DON-induced multigenerational toxic effects, and may be potential biomarkers for DON-affected molecular pathways.

3171 Using Caenorhabditis elegans for Predictive Oral Toxicity Assessment of Mixtures


The effects of exposure to individual toxic elements have been well studied. Information on the effects of exposure to mixtures of toxic substances is urgently required, however. Alternative toxicity models have the potential to provide relevant data for human safety assessment of chemicals with rapid tumourigenicity and at greatly reduced cost relative to traditional methods. Caenorhabditis elegans is a small nematode with a 3-day lifecycle that can be easily maintained in axenic liquid media using standard laboratory techniques. The C. elegans digestive tract has several features that are analogous to the mammalian digestive system, making this simple model organism an attractive candidate for predictive oral toxicity testing. Many pathways involved in organo-siloxanate development and neurotransmission are conserved from worms to people. C. elegans have consistently shown concordance for developmental toxicity or altered motor activity when exposed to mammalian developmental toxins or neurotoxins. We have designed a novel worm Development and Activity Test (wDAT) that maps the timing of C. elegans developmental milestone acquisition as well as stage-specific motor activity levels. Arsenic, lead, and mercury are mammalian developmental neurotoxins that have been associated with hyperactivity in children. The wDAT detected both developmental delay and hyperactivity for soluble salts of these toxic elements, indicating that worms and humans are concordant for these endpoints. One concern in mixtures risk evaluation is the in vivo occurrence of synergistic effects not predicted by in vitro testing or in silico analyses. Binary mixtures of arsenic, lead, and mercury produced equivalent or additive effects on developmental delay and/or hyperactivity, however no synergistic effects were detected. Mixtures with mercury produced the most significant additive effects. The wDAT used a collection of more than 400 transgenic C. elegans strains, each a transgene coding for a fusion product between nuclear-localized LET-858 and the additional target organ. Furthermore, multigenerational studies in mammals and aquatic animals looking at the offspring of exposed parents have demonstrated changes in the F1 generation, including altered neurodevelopment, growth, and oxidative stress markers. In the alternative model Caenorhabditis elegans (C. elegans) MCs induce germline apoptosis and decrease brood size at P0, making it an ideal model to study the epigenetic impact of MCs several generations. A multi-tiered approach is used to evaluate the transgenerational impact of MCs. Epigenetic histone modifications, including H3S10p and H3K9me3, in the germline are evaluated using immunofluorescence, germline stress is monitored through desilencing via loss of repressive histone regulation, and germline and somatic cell toxicity is evaluated through apoptotic, growth, and behavioral endpoints. L4s are exposed to a range of environmentally relevant concentrations MC-LR or MC-LF (0.1-100 μgL/L) for 48 hours with food in liquid. The C. elegans strains N2 (wildtype) and NL2507, carrying an integrated low-complexity, highly repetitive array composed of a transgene coding for a fusion product between nuclear-localized LET-585 and GFP (pks15680Let585:GFP; rol-6(su1006)), are used. For NL2507, the gene is transgenic in somatic cells but it is epigenetically silenced in the germline via accumulation of the repressive marks H3K9me3 and H3K27me3. Prior exposure studies using bisphenol A (BPA) have established this method to evaluate transgenerational toxicity. Exposed P0 worms are recovered at early and each generation is evaluated for multi- (F1 and F2) and trans- (F3+) generational toxicity without further MC exposure. This study helps establish the transgenerational reproductive and somatic cell toxicity of MCs and the role of phosphorylation in the epigenetic repression of the germline.

3172 Effects of Polybrominated Diphenyl Ether-47 (BDE-47) on Sensory Avoidance Behaviors in Caenorhabditis elegans

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Manufacturing of PBDEs was phased out from 2008–2014, due to their potential health effects and ubiquitous presence in air, soil, and water. Concern for human exposure remains the highest for infants and toddlers as studies have shown a correlation between increased blood PBDE levels and delayed cognitive development. BDE-47 is one of the most commonly found PBDEs in humans, especially young children whose hand mouth behavior increases ingestion of contaminated house dust. While studies suggest that exposure to PBDEs may impact cognitive and motor development, the sensory system has not been thoroughly examined as a possible target. This current study examined the effects of polybrominated diphenyl ether-47 (BDE-47) on sensory behaviors in Caenorhabditis elegans (C. elegans). AFD and ASH are major sensory neurons in C. elegans involved in thermo-sensation (AFD, ASH) and chemosensation (ASH). Wall and brood sizes of worms treated at early developmental larval stage 1 with BDE-47 (5µM, 10µM, 20µM) will present atypical sensory osmotic- and thermo-avoidance behavior. Gravid adult worms were synchronized and embryos were stored in M9 buffer 18-21 hours until hatching. L1 worms were treated with BDE-47, and plated onto freshly seeded plates. Plates for the osmotic avoidance assay contained a 2 centimeter diameter ring of 4M fructose in 1% congo red. Five nematodes were placed in the center of the ring, and the number of worms leaving the ring, touching the ring, or staying in the ring was recorded at 5 and 10 minute intervals. Worms that leave the ring are considered to display abnormal avoidance behavior indicative of sensory deficits. Data revealed a dose dependent effect with the control worms having the lowest percentage of worms leaving the ring and BDE-47 (20µM) treated worms having the largest percentage of worms leaving the ring. The thermal avoidance response assay was done with an electronically heated metal tip pen that was held 1 mm from the animals’ head for 3-5 secs. Their response to the extreme temperature change was grouped into four classes. Class I was a normal rapid reflex with direction change, class II was rapid reflex with only little backing motion, class III was a slow backing, and class IV was no response. Results showed, control groups had high numbers of class I and II responses while, 10µM and 20µM BDE-47 induced higher numbers of worms in class III and IV. Together the data indicates that PBDEs can alter sensory neuron development in an animal model and therefore could potentially be an environmental contaminant that may be linked to atypical sensory deficit disorders.
The acute toxicity of 45 chemicals was assessed using TruLarv™ (Galleria mellonella wax moth larvae), a non-animal technology (NAT) model and the results compared with data published in the OECD 129 guidance for two cytotoxicity test methods (3T3 NRU and NHK NRU) and published rodent LD₅₀ values. Test compounds utilised in this study were selected directly from the OECD 129 guidance document with chemical classes represented for organic compounds including carboxylic acids, heterocyclic compounds, alcohols, amides, phenols and selected inorganic compounds. Treatment was performed by subcutaneous microinjection of 10 μL test chemical into the front pro-leg. All treated and untreated control larvae were incubated at 20 - 25°C and scored for morbidity at 24 hours, 48 hours and 72 hours post challenge and LD₅₀ (mg/kg) values calculated. TruLarv™ LD₅₀ values obtained in this study were categorised in terms of hazard class based on the Globally Harmonized System (GHS) for classification and labelling of chemicals. For chemicals tested in this study where published rodent acute oral LD₅₀ values were >2000 mg/kg (category 5/low toxicity), the results in TruLarv™ showed 100% concordance with chemicals being classified in this same hazard category. In comparison, concordance of cytotoxicity methods with rodent acute oral LD₅₀ values was <50% with chemicals including acetalaminophen, citric acid, dibutylyltartrate, sodium hyochlorite, trichloroacetic acid and dimethyldfoformamide being predicted as weakly toxic or very toxic (category 1 - 4), reflecting the general tendency for cell culture systems to over-estimate the toxicity of chemicals, especially those of low toxicity. In conclusion, the results obtained in this study showed the TruLarv™ model to be superior to current in vitro cytotoxicity methods for predicting low-toxicity chemicals thereby, potentially providing a more predictive starting point for in vivo toxicity testing. Addition of this model to ECHA guidance as a recommended element in in vitro approaches could lead to a reduction in numbers of animals used for toxicity testing and strengthen the options for registrants to justify the waiving of in vivo acute oral toxicity studies for chemicals predicted to be of low toxicity.

Atrazine, a triazine herbicide, is one of the widely used herbicides in the United States and many other countries. Several epidemiological studies have associated atrazine (herbicide) exposure with increased incidence of type 2 diabetes (T2D), which accounts for more than 90% diabetic cases. Therefore, we used Drosophila melanogaster, having conserved Insulin/Insulin growth factor-like signaling (IGS) as well as glucose homeostasis, to identify the diabetogenic potential of atrazine and to delineate the mechanisms underlaying atrazine mediated onset of diabetes. Flies reared on food containing atrazine (2 or 20 μg/mL) from egg to adult display insulin resistance with hyperglycemia, hyperinsulinemia and hyperlipidemia hallmarks of type 2 diabetes. In addition, we found elevated levels of advanced glycation end products (AGEs) and the receptor for AGE (RAGE) in flies from exposed group. Further, we found the levels of reactive oxygen species (ROS) and phosphorylation of c-Jun N-terminal kinases (JNK), were significantly increased suggesting oxidative stress-mediated activation of JNK signaling in response to atrazine exposure. Mitigation of oxidative stress through over-expression of sod2 in atrazine (20 μg/mL) exposed flies, revealed significantly decreased ROS levels and reduced phosphorylation of JNK. Moreover, glucose and Akt phosphorylation levels in sod2 overexpression flies exposed to atrazine were comparable to those of controls, suggesting restoration in insulin sensitivity. Also, these flies had AGE and RAGE levels comparable to those in controls. These findings suggest that oxidative stress and JNK signaling activation in response to atrazine exposure might lead to insulin resistance condition. Finally, this study not only provided experimental evidence to the epidemiological propositions on the diabetogenic potential of atrazine but also highlighted the potential of Drosophila as a model for deciphering the mechanisms underlying xenobiotic mediated onset of type 2 diabetes.

Developmental Exposure of Zebrafish (Danio rerio) Larvae to ∆9-Tetrahydrocannabinol and Cannabidiol Causes Significant Transcriptomic Changes

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Countries are relaxing laws regarding, and like Canada legalizing, the use of cannabinoids such as ∆9-tetrahydrocannabinol (THC) and cannabidiol (CBD). Recently, US FDA approved Epidiolex (> 99% CBD) for treatment of developmental and pediatric epilepsy in children as young as 2 years old. As a consequence of their increased use, understanding potential adverse outcomes following exposure to cannabinoids during critical developmental periods is important. The objective of this experiment was to assess transcriptomic effects following a developmentally relevant exposure to THC or CBD. Zebrafish were waterborne exposed from 5 hours post fertilization (hpf) through the larval stage (96 hpf) to 0.42 ± 0.13 mg/L THC or 0.031 ± 0.025 mg/L CBD. THC and CBD concentrations were selected to be below the lowest observed adverse effect concentrations for developmental toxicity. RNA sequencing (RNA-Seq) was conducted on zebrafish larval brain and revealed differential expression of 1026 and 90 genes for CBD and THC, respectively. Among them, 351 genes were common to both treatment groups. Analysis of THC and CBD differentially expressed genes by Ingenuity Pathway Analysis (IPA) revealed that molecular pathways including hepatic effects, lipid/fatty acid metabolism, cell survival/viability and immune response were significantly increased, while pathways relating to inflammation were significantly decreased. For the 351 overlapping genes, the top two significantly modulated canonical pathways were FXR/RXR and PXR/RXR. IPA upstream regulator analysis identified molecules including IL6 and HNF4-A as potentially responsible for observed gene expression changes. IPA predicted significant toxicological effects related to the liver, kidneys and heart. Collectively, these results indicate developmental exposure to THC or CBD alters metabolism, hepatic, and immune function gene expression that may be linked to latent adverse outcomes. Supported by the National Institute on Drug Abuse R21DA044473-01.

Oxidative Stress Mediated JNK Signaling Activation Causes Insulin Resistance in Atrazine-Exposed Drosophila

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Atrazine, a triazine herbicide, is one of the widely used herbicides in the United States and many other countries. Several epidemiological studies have associated atrazine (herbicide) exposure with increased incidence of type 2 diabetes (T2D), which accounts for more than 90% diabetic cases. Therefore, we used Drosophila melanogaster, having conserved Insulin/Insulin growth factor-like signaling (IGS) as well as glucose homeostasis, in addition to other MTJ factors, in muscle development and function of Drosophila. We therefore hypothesized that MeHg disrupts muscle development by targeting the myotendinous junction (MTJ). To address this hypothesis, we evaluated morphological and functional phenotypes following larval exposure to increasing concentrations of MeHg. In parallel, we assessed phenotypes of flies carrying RNAi knockdown constructs of genes involved in MTJ formation, including kon. Indirect flight muscle (IFM) morphology during pupal development was visualized using fluorescent reporter fly lines and immunostaining, while functional assays examined eclosion (emergence of adults) and flight behavior. MeHg treatment was seen to induce a dose-dependent increase in myosin heavy chains within developing dorsal longitudinal bundles of the IFMs, which paralleled a decrease in eclosion. Targeted RNAi knockdown of kon was seen to cause hyperactivity of the IFMs, while knockdown of kon and HNF4A as potentially responsible for observed gene expression changes. The top two significantly modulated canonical pathways were FXR/RXR and PXR/RXR.
Development of a Zebrafish S1500+ Sentinel Gene Set for High-Throughput Transcriptomics


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Sentinel gene sets have been developed with the purpose of maximizing the information from targeted transcriptomic platforms. We recently described the development of an S1500+ sentinel gene set which was built for the human transcriptome, utilizing a data and knowledge-driven hybrid approach to select a small subset of genes that optimally capture transcriptional diversity, correlation with other genes based on large-scale expression profiling, and known pathway annotation within the human genome. While this detailed bioinformatics approach for gene selection can in principle be applied to other species, the reliability of the resulting gene set depends on availability of a large body of transcriptomics data. For the model organism zebrasfish, we aimed to create a similar sentinel gene set for zebrafish (zS1500+ gene set), however, there is insufficient standardized expression data in the public domain to train the gene correlation model. Therefore, our strategy was to use human-zebrafish ortholog mapping of the human S1500+ genes and nominations from experts in the zebrafish scientific community. Knowledge from three ortholog databases was combined to determine human-zebrafish orthology. NCBi HomoloGene, Zebrafish Information Network (ZFNN), and Ensembl. 2,283 human S1500+ genes (84%) were found to have zebrafish orthologs. Prioritizing expert-nominated zebrafish genes (allowing multiple zebrafish orthologs for some human genes), 2,849 zebrafish orthologs of the human S1500+ genes were placed on the list. 206 additional expert-nominated genes were included for a total of 3,055 genes on zS1500+. Here we present the bioinformatics curation and refinement process to produce the final zS1500+ gene set, explore whole transcriptome extrapolation using this gene set and assess pathway-level inference. Leveraging the human-zebrafish ortholog mappings and a zebrafish > human > zebrafish extrapolation technique, we were able to approach 85% of the zebrafish whole transcriptome. This gene set will be immediately useful in performing targeted high-throughput transcriptomics in zebrafish for toxicogenomic screening and other research domains.

Leveraging Zebrafish to Identify Chemicals Disrupting Early Embryonic Development

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Alternatives to conventional toxicity testing are needed to support chemical screening and prioritization. Zebrafish embryos offer one of the most promising cost-effective vertebrate models to support toxicity testing. Therefore, the overall objective of this project was to leverage early, non-protected life stages of zebrafish embryos to identify compounds that disrupt the normal trajectory of early embryonic development. For the first aim, we carried out a high-content screen of the LOPAC2800 (Library of Pharmacologically Active Compounds) library - a commercially available library of 1,280 marketed drugs, failed development candidates, and well-characterized small molecules widely used for validation of high-throughput screening assays. Based on this screen, niclosamide - an anthelmintic drug used worldwide for the treatment of tapeworm infections - was one of the most potent development toxicants within zebrafish embryos during the first 25 h of development, with exposure to 10 μM niclosamide from 2-25 h post-fertilization (hpf) resulting in 100% embryo mortality. Therefore, the second aim of this study was to investigate the mechanism of toxicity of niclosamide during early stages of embryonic development. We found that niclosamide induced a concentration-dependent delay in epiboly progression during late-blastoula and early-gastrula stages that was dependent on exposure during the maternal-to-zygotic transition - a period characterized by zygotic genome activation and initiation of cell motility. Moreover, we found that niclosamide did not affect embryonic oxygen consumption, suggesting that oxidative phosphorylation - a well-established target for niclosamide within intestinal parasites - may explain a role in niclosamide-induced epiboly delay. However, mRNA-sequencing revealed that niclosamide exposure during late-blastoula and early-gastrula resulted in an increased abundance of maternal transcripts and decreased abundance of zygotic transcripts at 5 hpf relative to time-matched vehicle controls, suggesting that niclosamide may delay maternal transcript degradation and zygotic genome activation and may be preventing the progression of epiboly by disrupting the timing of the maternal-to-zygotic transition. Overall, our findings highlight the utility of embryonic zebrafish as a physiologically-intact, non-mammalian model for screening and prioritization of chemicals and environmental samples for further testing within rodents and human cell-based systems.

Coupled Host and Microbial Responses to Pthalate Exposure: Implications for Obesity


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The prevalence of obesity has increased dramatically over the past 40 years at a rate no explained by poor diet and lack of exercise alone. Environmental chemical contaminants that are suspected to contribute to the obesity epidemic are called obesogens. Epidemiological evidence points to phthalates as a suspected class of obesogens to which humans are exposed daily due to their use as plasticizers in various products, including plastic packaging, medical devices, and toys. Mechanisms by which phthalates, such as diethylhexyl phthalate (DEHP), contribute to exacerbated obesity are largely unknown and focus on the host. However, the gastrointestinal microbiome may also undergo changes following phthalate exposure that contribute to the obese phenotype. This study addresses the contributions of host and microbial alterations to exacerbation of obesogenic mechanisms in zebrafish exposed to DEHP (Dano rio). Adult zebrafish were randomly assigned to one of three feeding regimes for 60 days: 1) Normal feeding (10mg/kg fish), 2) Overfeeding (20mg/kg fish), or 3) Overfeeding with DEHP exposure at 3 mg/kg food. Mass and body mass index (BMI) were measured throughout the duration of the experiment, and at 60 days zebrafish were euthanized and tissues and fecal matter excised for RNAseq, qPCR, and 16s microbial sequencing. Overfeeding and overfeeding with DEHP elevated BMI compared to normal feeding but differences in BMI at 60 days were not observed between these two groups. Significant alterations in both host and microbial processes were exacerbated in the DEHP treatments. In the host, processes related to lipid metabolism, immune system function and gut physiology were altered following DEHP exposure compared to overfeeding alone, and the emergence of peroxisome proliferator activated receptor alpha as a potential modulator of these processes is noted. The gastrointestinal microbiome experienced dysbiosis characterized by decreases in Bacteroidiae, traditional and in Fusobacteria taxa following only the DEHP exposure. Furthermore, DEHP treatment resulted in significant changes in bacterial carbohydrate, galactose, inositol, phosphorus, and taurine and hypertaurine metabolites. Finally, co-occurrence network analysis revealed decreases in cluster size and a fracturing of the network into unconnected communities following DEHP treatments. These findings suggest a role for DEHP in exacerbation of obesogenic mechanisms following overfeeding through both host gene expression alterations and microbial dysbiosis.
3182 The Results of a Pilot Study Applying Systematic Review Methods to Toxicological Test Method Assessment

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Systematic reviews (SR) involve a series of steps including framing the review question, defining inclusion and exclusion criteria, building a high-quality search strategy, assessing the risk of bias, extracting data, and analyzing and interpreting the results. SR play an important role in clinical medicine due to their transparency, objectivity, and structured methodology. Within toxicology, systematic reviews have not yet been applied to test method performance. In this study, conducted by a multi-stakeholder workgroup, a systematic review was performed to investigate the Zebrafish Embryological Toxicity Test (ZET) as a possible method for informing the assessment of developmental toxicity. The systematic review was designed to compare the outcomes of ZET and standard mammalian toxicity tests for the same set of substances. A two-stage literature search was conducted to (1) find ZET studies of well-defined substances and (2) find mammalian developmental toxicity studies that tested the same substances. The ZET search resulted in 5,074 studies. Of these, 50 were randomly selected for a preparatory study. After screening the titles and abstracts against predefined criteria, eight studies were included. After screening the full texts of these eight studies, one study was included in which seven substances were tested. We systematically searched the mammalian literature for these seven substances, which resulted in 1,442 studies. These were reduced to 263 studies after title and abstract screening and to 12 after full-text screening. These 12 studies contained data on two of the previously identified seven chemicals: thalidomide, tested in both rats and rabbits, and gambogic acid, tested only in rats. This preparatory study proved that translating systematic review methodology to a toxicological test method assessment is feasible, and we learned lessons along the way that helped us operationalize this process.

3183 Multi-omic Approach to Inform Quantitative Adverse Outcome Pathway Development for Acute Organophosphorus Poisoning

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Organophosphorus (OP) compounds constitute a class of acetylcholinesterase (ACHE) inhibitors used as not only pesticides but also chemical warfare nerve agents. Acute OP poisoning (OPP) can result from occupational (agriculture, industry, research), accidental, suicidal or homicidal causes and can be graded by severity as mild, moderate and severe. Epidemiological studies regarding OP pesticides estimate approximately 3 million cases of severe OPP and 300,000 deaths annually, most of them in developing countries of the Asia-Pacific region. Recently, we generated zebrafish models for mild, moderate and severe OPP. These were reduced to 263 studies after title and abstract screening and to 12 after full-text screening. These 12 studies contained data on two of the previously identified seven chemicals: thalidomide, tested in both rats and rabbits, and gambogic acid, tested only in rats. This preparatory study proved that translating systematic review methodology to a toxicological test method assessment is feasible, and we learned lessons along the way that helped us operationalize this process.

3184 Alterations of Expression of RNA Modification Regulators by Carcinogens in the Alternative Chicken Egg Model

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The abundance of cellular RNA structural modifications is well documented. Such epitranscriptome alterations ultimately regulate the expression of genes that control many biological processes. However, their role in various pathologic conditions, including cancer carcinogenesis in the developing organism, remains unclear. Similar to epigenetic alterations, epitranscriptome modifications are dynamic and may control enzymes, including methyltransferases, pseudoU synthetases and demethylases. The current study used microarray platform to investigate presence and expression of genes which encode for aforementioned enzymes in the Chicken Egg Model (CEM) after 3 daily injections of a wide array of established genotoxic and epigenetic carcinogens and their non-genotoxic comparators, including aromatic amines, dialkyltinotcarnosines, polycyclic aromatic hydrocarbons, aflatoxins, clofibric acid and phenobarbital. The CEM is an alternative to animal models that uses chicken fetal livers collected 3 hours after the last dosing on the incubation day 11, to evaluate various effects of chemicals, including their potential to produce DNA damage, alterations in gene expression and histologic changes. Chicken embryo-fetus is an intact, developing, metabolically active organism, which by definition is not yet subject to regulation for animals. Chemical-specific deregulation of 15 genes which encode for several RNA modification enzymes in other species including humans, in CEM could be expected by the presence of these enzymes in cell lines. Diallylthioleminosines produced most significant changes in the expression pattern of detected genes. Additionally, the majority of genotoxic carcinogens from the selected set of compounds altered the expression of several small nucleolar RNAs, including SNORD17, SNORAD2 and SNORA 18, which guide methylation and pseudouridylation of primarily RNA and tRNA. These findings lead to the hypothesis that chemicals are capable of producing chemical modifications of RNA, which in combination with established genotoxic and/ or epigenetic DNA damaging effects contribute to the process of chemical carcinogenesis. Based on our findings, CEM is an appropriate model to investigate this hypothesis.

3185 DNA Damage Produced by α- and β-Asarones in Alternative In Ovo Genotoxicity Models

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The genotoxicity of several representatives of the food-borne alkenylbenzene (AB) class was previously reported in two in ovo genotoxicity models, the Turkey and Chicken Egg Genotoxicity Assays (TEGA and CEGA). In the current study, two additional ABS, α- and β-asarone, found in plants, e.g. Acorus and Asarum were investigated in both TEGA and CEGA for genotoxicity using comet and 8-<sup>P</sup>-postlabeling (NPL) assays. Additionally, fetal turkey liver samples were analyzed with ultra-high performance liquid chromatography (UHPLC)-electrospray ionization (ESI)-tandem mass spectrometry (MS/MS) method for confirmation, quantification and characterization of DNA adducts. DNA adducts detected with NPL assay. Fertilized turkey or chicken eggs were incubated under TEGA or CEGA protocol with vehicle, several dose levels of either α-asarone (5,10 mg/egg in TEGA; 2,4,8 mg/egg in CEGA) or β-asarone (2.5, 5 mg/egg in TEGA). Negative comet findings for β-asarone in CEGA could be explained by lower doses tested in chicken eggs, due to higher toxicity of the compound to chicken fetuses compared to that in turkeys. Both, α- and β-asarone also produced DNA adducts detected by NPL in chicken and turkey fetal livers. The chromatographic pattern of adducts formed by asarones was distinct for each compound while being similar between CEGA and TEGA. UHPLC-ESI-MS/MS analysis confirmed the presence and the nature of adducts after exposure to both ABS. In all samples the amount of dG adducts exceeded the amount of dA adducts. Thus, our findings, congruent with other data provide evidence of genotoxic potential for α- and β-asarones. Moreover, extensive analyses of DNA adducts formed in the in ovo models validated the sensitivity of NPL assay as a part of genotoxicity assessment in ovo. Overall, our results confirm that TEGA and CEGA can be used interchangeably.
Detection of Dose-Response Effects and No-Adverse-Effect-Levels of DNA Adduct Formation by Genotoxic Carcinogens in the Turkey Egg Genotoxicity Assay

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Increased understanding of cellular defense mechanisms for DNA damage challenges the hazard based “as low as reasonably achievable” (ALARA) concept, and indicates that the existence of thresholds for genotoxic carcinogens is a valid assumption. Here we utilize the Turkey Egg Genotoxicity Assay (TEGA) to investigate and report on dose-response effects and the possibility of no-adverse-effect-levels (NOAELs) for the formation of DNA adducts of three known genotoxic carcinogens: 2-Acetylaminofluorene (AAF), Benzo[a] pyrene (BaP), and Quinoline (QUI). Medium white turkey eggs with 22- to 24-day old fetuses received 3 daily injections of a wide dose range for each compound: AAF (0.0005 - 0.05 mg/egg), BaP (0.05 - 0.3 mg/egg), and QUI (0.039 - 10 mg/egg), livers were harvested 3 hours after the final injection and the 32P-nucleotide postlabeling (NPL) assay was utilized to detect adducts in the fetal turkey livers. All materials produced DNA adducts in a dose-related manner. Even though very low doses were tested, a threshold level was not identified for AAF, the most potent hepatocarcinogen evaluated in these experiments. This finding highlights the sensitivity of the turkey liver to DNA damage produced by AAF as well as the sensitivity of the NPL method in general. NOAELs for DNA adducts were detected for BaP (0.15 mg/egg - 4 mg/kg bw) and QUI (0.08 mg/egg - 2 mg/kg bw), demonstrating that safe exposure levels to genotoxic compounds can exist and should be considered in the risk assessment of such compounds.

The Necessity of Uncertainty: Quantifying Uncertainty for Regulatory Application of New Approach Methodologies

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The face of regulatory toxicology is undergoing a makeover in the 21st century with the encouragement of new approach methodologies. However, is the way the scientific community accesses and understands uncertainty adapting to developing methodologies? Historically, toxicology testing for the assessment of human health has used animals, in which uncertainty factors were incorporated to address issues such as interspecies differences and species-specific mechanism of action. These uncertainties are in addition to the standard scientific uncertainties that exist with any experimental data. With the international push to accept alternative nonanimal testing in lieu of traditional in vivo testing, now is the time to understand acceptable uncertainty for developing methodologies. However, if uncertainty cannot be assessed and addressed in the same manner as the scientific community is comfortable with for in vivo data, what levels and expressions of uncertainty would be acceptable to make a regulatory decision with a new approach methodology for a specific regulatory context? This roundtable will discuss how uncertainty has historically been quantified, how it differs between methods (in vivo/in vitro/in silico), and how it has been addressed and should be addressed in order to facilitate development and implementation of new approach methodologies. We dare to broach the cultural barriers to accepting new approach methodologies in safety and risk assessment. We also intend to incite dialogue to address what types and subsequent levels of uncertainty are likely or tolerable in 21st-century toxicology.

Toxicology Education and Risk Assessment Training in Africa: Status, Challenges, and Role of SOT Special Interest Groups in Moving Forward

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Reliable information about formal toxicology curricula and education-training in Africa is very scarce. Illustrative of that example is the fact that in the last 20 years there has been few publications (fewer than five) that have discussed toxicology education in South Africa and veterinary toxicology education in Africa. The continent of Africa is in a stage of rebirth after a long time in stagnation. Africa is now home to seven of the world’s 10 fastest growing economies. Africa is very rich in human capital, and today nearly 50% of Africans are under age 15. Africa has the fastest growth-rate in the world (due to dropping child mortality and high fertility), with the continent expected to have an estimated 2.8 billion people by 2060. Overall, the education in Africa is slowly improving. However, there is outstanding improvement in children’s primary education. The number of children enrolled in primary schools more than doubled, from 62 million to 149 million children, within 22 years (1990–2012). A systematic review indicates that university education continues to pose a significant challenge in Africa. In the continent’s 10 most-populous nations, there are 740 universities serving some 660 million Africans. This ratio in terms of the number of universities compared to the US represents a week 10%, with the US has some 5,300 universities and colleges serving a population of over 323 million people. Historically Africa hosts the two oldest universities in the world: the University of Al Qarawiyyin in Fez, Morocco, which opened in AD 859, and Al-Azhar University in Egypt, part of the larger complex of institutions associated with the Al-Masjid Mosque and Library. Today, African institutions enroll nearly 2 million students. This roundtable will provide an overview of the educational and training challenges within the toxicology diaspora and present research findings from studies that can inform the approach that SOT Special Interest Groups (SIGs) take to address the dilemma going forward. Desired outcome: is the majority of presenters have been involved in or are developing the toxigology and risk assessment training in various parts of Africa, the presenters will share this experience and explore innovative strategies to address the educational and training curricula in Africa despite the lack of meaningful investment in educational and research infrastructure by most African governments. A summary of the session presentations and recommendations will be shared with various organizations working in Africa.

Stepping Out of the Lab: Maximizing Access and Experience for Internships in Toxicology

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A major goal of the SOT Education Committee is to expand opportunities for students to engage in internships within industry, government, and nonprofit organizations. Immersion in internships provides students unique appreciation of the day-to-day activities of toxicologists and paths for success in these sectors. Such opportunities can help students establish their professional network, build confidence in career choices, and ultimately better prepare for transitioning into these sectors upon graduation. While numerous industries and governmental agencies have internship programs, or comparable opportunities, identifying these opportunities can be challenging. There are also barriers associated with funding, mentor buy-in, timing of the internship relative to the student’s graduate training, and feasibility for international student participation. The goal of this session is to bring together the various stakeholders—graduate students, faculty, and those hosting interns—to discuss best practices for developing internships, and strategies for increasing the number of available internships as well as improving awareness and access to available internships. The session will consist of five presentations followed by a group discussion on strategies that the SOT Graduate Education Subcommittee might develop to increase internship opportunities and overcome barriers for industry and government to host interns, and for graduate students to attain highly competitive internships. The formal talks will begin with a presentation by an individual who completed an internship as a graduate student discussing the benefits and logistical challenges they currently experience. Following this, representatives from various organizations working in Africa will share this experience and explore innovative strategies to address the educational and training curricula in Africa despite the lack of meaningful investment in educational and research infrastructure by most African governments. A summary of the session presentations and recommendations will be shared with various organizations working in Africa.

Consideration for Safety Assessment of Chemically Synthesized Therapeutic Peptides: A Drug Development Paradigm between the Large and Small

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Thousands of naturally occurring peptides act as key signaling molecules and thereby regulate crucial physiological pathways. Due to their high target selectivity and attractive intrinsic properties, novel chemically synthesized peptides continue to gain interest as an effective modality against drug-resistant targets. Currently, over 60 therapeutic peptides have gained worldwide approval and approximately 150 candidates are in clinical development. As with any other novel technology, development and safety assessment of chemically synthesized peptides present with an array of complex challenges. Additionally, there is a lack of specific regulatory guidance for peptide development. To address these challenges, this session will have presentations in preclinical (ICH M3(R2) and biologic (ICH S6(R1)) guidelines to develop therapeutic peptides. This symposium has been designed to provide a general scientific and strategic framework for safety assessment of therapeutic peptides. The session will begin with an introduction to peptides and give insights to their history,
Oxidative stress is an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling, which in turn leads to cellular injury. Damage mediated by reactive oxygen species (ROS) can arise from endogenous sources, such as mitochondria and peroxisomes, or by exogenous sources, such as pollution or tobacco smoke. ROS can cause DNA damage, lipid peroxidation, and protein oxidation, leading to cell death or cellular transformation.

In recent years, oxidative stress has been implicated in a variety of diseases, including diabetes, cardiovascular disease, and cancer. Oxidative stress is also associated with aging and neurodegeneration. Therefore, it is important to understand the mechanisms of oxidative stress and develop strategies to mitigate its effects.

The potential for oxidative stress in synthetic peptide drug development must be considered. Peptides may contain reactive groups or be modified to enhance membrane permeability, which can increase the risk of oxidative stress. Additionally, impurities in the synthetic peptide drug product may also contribute to oxidative stress. Therefore, it is important to assess the potential for oxidative stress in peptide drug development and to develop strategies to mitigate this risk.
ROS-Mediated Epigenetic Changes in Developing Lungs

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Reactive oxygen species (ROS) are essential to many physiological processes including reproduction. An imbalance of redox homeostasis can occur during pregnancy resulting in premature birth. Room air represents a “hyperoxic” environment to the preterm infant that developmentally should be in the low oxygen environment of the uterus. Furthermore, preterm infants are often treated with elevated oxygen tension to support gas exchange in underdeveloped lungs. The combination of increased oxidant stress from the cause of preterm birth and treatment with elevated oxygen tension, results in increases in ROS concentrations which can promote epigenetic modifications in the offspring. Increases in ROS concentrations are associated with increased activity of methyl transferases resulting in greater methylation of CpG islands and histone proteins. In human and mouse studies, we have identified increases in enzymes responsible for DNA methylation (DNMTs) and decreases in enzymes responsible for histone protein methylation (PRMTs) in immature hyperoxia-exposed murine lungs. Further, we have seen repression of histone methylation sites H3K9, H3K27, H4R3 which are responsible for transcriptional repression. This presentation will offer current data on changes in methylation and its role in altered lung development and new theories on treatments with demethylases as a novel therapeutic approach.

NRF2, Oxidative Stress, and Inflammatory Lung Injury

D. Zhang, University of Arizona, Tucson, AZ.

The transcription factor NRF2 is a critical regulator of cellular redox homeostasis whose target gene expression requires NRF2-SMAF dimers binding to antioxidant response elements (AREs) to initiate transcription. NRF2-ARE-dependent transcriptional repression is unreported, however, our group recently described a novel NRF2-mediated repression of the MYLK gene, which encodes the central cytoskeletal effector non-muscle myosin light chain kinase isoform (nmMLCK), via a 7 nucleotide NRF2-negative regulatory sequence (NNRS) adjacent to the ARE. We identified replication protein A1 (RPA1) as a novel binding partner of NRF2 that outcompetes NRF2-sMAF for ARE binding to reduce MYLK promoter activity. Furthermore, genome-wide silico and RNAseq analysis revealed that the NRF2-RPA1-ARE-NNRS complex also suppresses the expression of other genes involved in a diverse array of cellular processes, including tumor suppression (RASSF10 and FOCAD), cell growth and proliferation (FAM110B and NAV2), cell-cell adhesion (ITGA1 and TANC2), vesicle transport (SYT16), and the immune response (ADGRG5).

Finally, we investigated NRF2-mediated suppression of target genes by investigating MYLK repression in cellular processes, including tumor suppression (RASSF10 and FOCAD), cell differentiation, and inflammation. The symposium will also discuss new opportunities for translational research, which leads to the development of rational strategies (e.g., discovery of novel drugs in collaboration with pharmaceutical industry) for the prevention and/or treatment of human diseases by oxidative stress. Thus, this symposium would be of great interest to toxicologists who are focusing on the mechanisms of toxicity of a wide variety of chemicals that mediate toxicity by mechanisms entailing oxidative stress.

Mechanistic Role of Cytochrome P4501A and 1B1 Enzymes in the Metabolism of Reactive Oxygen Species (ROS)-Mediated Formation of Lipid Hydroperoxides: Implications for Hyperoxic Lung Injury and Human ARDS

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Hyperoxia is frequently used in the treatment of pulmonary insufficiency in preterm and term infants and in adults with acute respiratory disease (ARDS). In preterm infants, hyperoxia contributes to the development of broncho-pulmonary dysplasia (BPD). The molecular mechanisms of oxygen-mediated pulmonary injury are not understood, but reactive oxygen species (ROS) are the most likely candidates. Using in vivo approaches entailing Cyp1a1-null, new mechanism of NRF2 function, and highlight the potential importance of targeting NRF2-mediated suppression of target genes as a therapeutic approach for treating lung pathologies.
Cyp1a2-null and Cyp1b1-null mice, and in vitro approaches entailing purified human CYP1A1/1A2 and LC-MS/MS, we observed novel roles for cytochrome P450 (CYP1A) enzymes in the metabolism of F2-isoprostanes, which are generated by ROS-mediated mechanisms. Mice lacking the gene for CYP1A1 and the liver-specific CYP1A2 are more susceptible to hyperoxic lung injury, and these mice displayed augmented levels of pulmonary F2-isoprostanes and isoforms, suggesting that these molecules contribute to hyperoxic lung injury. On the other hand, mice lacking the Cyp1b1 gene show lesser injury than WT mice, suggesting a pro-oxidant role of CYP1B1. When mice lacking Cyp1a1/b1 double null and Cyp1a1/a2/b1 triple null mice were exposed to oxygen, the phenotype was similar to Cyp1b1-null mice, suggesting that CYP1B1 plays a key role in oxygen-mediated lung injury. We also found formation of bulky oxidative lesions (oxidative DNA adducts) in tracheal aspirates of premature infants and adults who received supplemental oxygen, suggesting that these adducts could serve as new biomarkers of BPD and ARDS. Future studies focusing on development of CYP1B1 inhibitors, in collaboration with pharmaceutical companies, could lead to prevention/treatment against hyperoxic lung injury in humans. Thus, the research described above offers novel opportunities for the development of rational strategies for the prevention/treatment of lung diseases in humans associated with hyperoxia.

3202 Understanding the Utility of In Vitro Developmental Toxicity Assays and Building Integrated Testing Strategies
J. Palmer, Stemina Biomarker Discovery, Inc., Madison, WI.

The performance of many alternative methods for developmental toxicity testing has been evaluated over the last 20 years; however, their application as new approach methods (NAMs) in a regulatory setting is still poorly defined. Several groups and regulatory agencies are working on ways to address this limitation through the development of strategic roadmaps and updating current testing requirements, including ICCVAM, US FDA, US EPA, ICH, ECVM, JACVAM, and the EU-ToxRisk consortium. Given the numerous complex processes involved in fetal development, it is unlikely that a single assay or adverse outcome pathway (AOP) concept will be sufficient for understanding and/or predicting the developmental toxicity potential of chemicals. Defining the applicability domain of each NAM, in terms of both chemical and biological space, establishing scientific confidence in their validity, and characterizing how they are best used in integrated testing strategies will be key for gaining regulatory acceptance of alternative methods. Evaluating well-defined groups of reference chemicals, such as the list proposed in the draft ICH Guideline SS(R3) on Detection of Toxicity to Reproduction for Human Pharmaceuticals or environmental chemicals identified by ICCVAM with robust animal data in multiple species, can help provide insight into the limitations of NAMs and how they can be combined. Systematic review techniques can integrate large sets of information from the scientific literature to identify high-quality studies and develop a weight of evidence approach for the application of NAMs. This session will highlight how the use of reference chemicals, systematic review, and evolving validation practices can be used to define the utility of NAMs, to develop integrated testing strategies, and will inform the discussion on how these approaches can help the field move toward regulatory acceptance. Experts from industry, academia, and government will present the results from a broad spectrum of reference chemicals (pharmacological, agrochemical, industrial, etc.) evaluated with different NAMs to demonstrate how these can be used to define the applicability domain of an assay.

3203 Evaluation of the Reference Chemicals Suggested in the Draft ICH SS(R3) Guideline with a Human Pluripotent Stem Cell-Based Developmental Toxicity Assay
J. A. Palmer, Stemina Biomarker Discovery, Inc., Madison, WI.

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) recently released the draft SS(R3) Guideline on Detection of Toxicity to Reproduction for Human Pharmaceuticals. The revised guideline would allow the use of in vitro, ex vivo, and non-mammalian embryo and developmental alternative assays to replace or eliminate in vivo studies in certain circumstances and provides a framework for qualifying alternative test systems for regulatory acceptance. The draft guidance includes a list of reference chemicals aimed at defining the applicability domain of an alternative assay. This list of chemicals has been evaluated with the DEVTOX quickPredict (devTOXqP) assay, which is an in vitro human pluripotent stem-cell based assay that predicts the developmental toxicity potential of chemicals based on changes in hPS cell metabolism. The results from this evaluation, as well as how devTOXqP can be used with other NAMs in an integrated testing strategy will be discussed.

3204 Retrospective Analysis: Can Existing Literature Be Used to Compare the Results from the Zebrafish to Mammalian Embryotoxicity Tests?
K. Tsaiou, Johns Hopkins University Center for Alternatives to Animal Testing (CAAT), Baltimore, MD.

A multi-stakeholder working group was assembled to conduct a systematic review (SR) comparing the performance of developmental toxicity test methods. In one, OECD Test Guideline 414 (Prenatal Developmental Toxicity) in rats and rabbits, which is currently mandated by various regulatory agencies for chemical registration is compared to a potential alternative, the Zebrafish Embryological Toxicity Test (ZET). ZET is being investigated as a possible replacement for the OECD TG 414 in total or replacement of one species (i.e., rabbit). We executed a wide literature search in 2016 and conducted a SR of all (7,778) published studies that used the ZET to screen 1,466 chemicals. These results, after assessment and comparison for methodological performance, are then being compared with the systematically reviewed results of OECD TG 414. The protocol, literature and database search strategies, and the results of the pilot study and lessons learned will be presented. The advantages of using text-mining and machine learning technologies in searching and reviewing the literature will be highlighted. We conclude that ZET is a valuable tool with which to assess the available literature on test method performance. Lessons learned and obstacles to doing literature-based assessments of test performance will be discussed.

3205 Mining and Modeling ToxCast/Tox21 Data for Developmental Toxicity
T. Knudsen, US EPA/ORD, Research Triangle Park, NC.

Synthetic reconstruction of embryonic development can provide in silico models that can be used to translate in vitro data from new alternative methods into critical phenomena for developmental toxicity. Computational methods are uniquely positioned to capture this connectivity and provide a mechanistic approach to AOP elucidation and toxico logical assessment with less reliance on animal testing. In ToxCast, for example, approximately 1 in 6 chemicals of 1065 tested give an exposure-based prediction of teratogenicity as a human stem-cell based assay. Mining these data for broader relationships to in vitro bioactivity profiles, together with model specific correlations to molecular pathways and cellular processes that drive human embryology and development, can be used in a defined approach to testing and assessment. This presentation will highlight some of the challenges for science and technology development in determining the applicability domain of high-throughput datasets ToxCast/Tox21 in support of developmental hazard identification and characterization. Progress in translating these large datasets into human-predictive models of developmental toxicity will be demonstrated utilizing case studies tying the in vitro data and in silico models to fundamental principles of teratogenesis. This work does not reflect US EPA policy.

3206 EU-ToxRisk DART Case Study Evaluating a Chemical Series across Multiple NAMs

Valproic acid (VPA) is a classical teratogen, and a set of nine VPA analogues with varying in vivo developmental toxicity potential were tested in the mouse embryonic stem-cell test, zebrafish embryotoxicity test, CALUX assays, and an iPSC-derived neuroepithelial cell model (UKN1). These NAMs, and a number of developed toxicokinetic models can be used with other structure-based information to apply read-across predictions within this chemical class and compared to reference in vivo data to understand their respective utility. This presentation will discuss combining results from multiple NAMs for predicting the teratogenic properties and potency of a series of structurally related chemicals and how this information can be used to establish a framework of testing for regulatory applications.
Under the US Strategic Roadmap for Modernizing Safety Testing, an ICCVAM working group was formed focusing on developmental and reproductive toxicity, with representatives from various federal agencies. The DART WG is developing a scoping document of regulatory needs and decision contexts, as well as opportunities for use of in vitro alternatives. An overview of the results of this effort, incorporating input from all US federal agencies that either require or consider safety testing data for developmental toxicity, will be presented. The working group scope and charge includes not only creating a catalog of existing technologies, but also mapping new and emerging technologies to known mechanisms of developmental toxicity and relevant adverse outcome pathways. Work to map the ToxCast/Tox21 assay targets to developmental toxicity mechanisms, and use in chemical prioritization, will be described. Further, the group plans to use study data from the National Toxicology Program and curated literature studies are being used to identify environmental chemicals with developmental toxicities in multiple species, ranging from overt malformations to subtle effects, that could serve as reference chemicals for evaluation of NAMs. This talk will include a discussion of the challenges and opportunities in data extraction and standardization, and the resulting database and candidate reference chemicals.

Active smoking and exposure to second hand smoke (SHS) increase the risk of vascular disease and is a major public health concern, but the mechanism(s) of action are complex and not fully understood. Disruption of endothelial functional homeostasis is considered as a key event in the development and progression of atherosclerosis. Endothelial homeostasis encompasses acute responses, such as adaption of flow to tissue’s demand, and more sustained responses to injury such as re-endothelialization and sprouting of endothelial cells and attraction of endothelial progenitor cells (EPCs) both supporting repair of damaged endothelium and maintenance of arterial integrity. The balance and the intensity of endothelial damage and repair might be reflected by inverse changes in circulating endothelial microparticles (EMP) and EPCs. Smoking appears to tip the balance by both inflicting endothelial injury while blunting regenerative responses leading to endothelial dysfunction and mal-adaptive arterial remodeling. One of the mechanisms may be an inhibition of nitric oxide (NO) production either through nitric oxide synthase in endothelial cells or EPCs. Previous studies indicate that even brief exposure to real-world levels of second hand smoke leads to vascular injury with release of EMPs, sustained endothelial dysfunction, and blunting of EPC function. While EPCs are mobilized into circulating blood, these cells are dysfunctional due to impaired nitric oxide production likely involving cotinine but also particulate matter. Dissecting the individual pathophysiologial roles of components of ambient air pollution is timely as the use of e-cigarettes is becoming increasingly popular and global urbanization is rapidly progressing.

Exposure to ambient air pollution is a leading cause of death worldwide. It has been linked globally to 7 million premature deaths and $5 trillion in costs per year. With continuing industrialization and urbanization, this disease burden is expected to increase even further. Interestingly, the majority of air pollution-associated deaths are due to cardiovascular disease (CVD). It has been estimated that in 2016, exposure to ambient and household air pollution combined was responsible for 3.5 million cardiovascular deaths. Because of this, air pollution exposure is now recognized as a modifiable risk factor that contributes to cardiovascular morbidity and mortality. Regardless of the strong evidence that air pollution exposure increases the risk for developing CVD, it remains unclear how the exposure to polluted air induces cardiovascular injury. Previous work has shown that chronic exposure to polluted air is associated with decreased endothelial function, suggesting that long-term inhalation of air pollution might result in endothelial injury and dysregulation of vascular homeostasis—effects that could accelerate CVD or trigger adverse cardiovascular events. Current research suggests that vascular homeostasis and endothelium health are maintained, at least in part, by endothelial progenitor cells (EPCs). These cells are a subpopulation of proangiogenic cells that reside in the bone marrow and circulate in the peripheral blood. Upon hypoxia or vascular injury, EPCs are mobilized from the bone marrow and home to the site of tissue damage where they contribute to vasculogenesis and/or angiogenesis either through terminal differentiation into mature endothelial cells or by paracrine stimulation of wound healing processes. Interestingly, recent studies show that EPCs are early and direct targets of air pollutant exposure. For instance, air pollution-induced impairments in EPC number and function have been found in humans exposed to particulate or volatile air pollution and in controlled exposure studies in rodents. These exposure studies demonstrated that inhalation of polluted air affects both circulating and bone marrow EPCs. This is important because chronically low circulating EPC levels and EPC dysfunction have been associated with vascular dysfunction in the risk and severity of CVD. Moreover, treatments that improve the number and function of EPCs (e.g., exercise, antihypertensive drugs) attenuate cardiovascular dysfunction. Because of the critical and non-redundant roles of EPCs in vascular health, air pollution exposure-induced EPC depletion and dysfunction could disturb vascular maintenance and repair, impairing vascular function, and consequently increase the risk for CVD. Hence, addressing how air pollution exposure induces EPC depletion and dysfunction is of high significance because it would help to discern the specific mechanism by which exposure to polluted air increases the risk for CVD. Understanding such mechanisms is important to develop effective prophylactic interventions and evidence-based regulations to mitigate against the major harmful health effects of air pollution. This session will highlight human studies and animal research that investigate the effects of air pollution exposure on EPCs. The specific presentations of the session will show that (1) inhalation of secondhand smoke modulates EPC number and function in healthy nonsmokers; (2) acute exposure to increased levels of ambient particulate matter (PM2.5) or other exposure to volatile organic components (benzene, acrolein) of polluted air are associated with changes in the number of these circulating proangiogenic cells; (3) the exposure to metal-rich particles impairs circulating EPC levels in humans and decreases number and function of bone marrow derived EPCs in mice; and (4) exposure to concentrated PM2.5 (CAP), by inducing oxidative stress, impairs circulating EPC levels and induces an anti-angiogenic dysfunctional EPC phenotype with a reduced ability to promote vascular tissue repair. Taken together, this session will provide a comprehensive overview that summarizes novel aspects of the mechanistic insights in understanding the adverse effects of air pollution exposure on cardiovascular health associated with changes in EPC number and function.

Exposure to airborne particulate matter (PM2.5) and volatile pollutants impacts the levels of circulating angiogenic cells in humans.

In response to vascular damage or hypoxic signals, a subset of adult progenitor cells, the circulating angiogenic cells (CACs), are mobilized from storage niches, home to sites of injury and participate in vascular regeneration through terminal differentiation or paracrine effects. Indeed, several animal studies have demonstrated the utility of CACs in restoring vascular perfusion and tissue repair. Levels of CACs are thus a useful indicator of vascular health and are inversely correlated with adverse outcomes. However, the availability of CACs is highly responsive to external cues and their levels are influenced by concurrent disease, lifestyle choices, and environmental exposures. In particular, we have shown that the levels of several CAC subtypes (most prominently those of the CD34+/CD31+/CD45+/CD133+ phenotype) are decreased in young healthy adults in response to acute exposure to fine airborne particulate matter (PM2.5). Consistent with this depletion of CACs and impairment of vascular perfusion, we also found that exposure of the same cohort led to a decrease of pro-angiogenic growth factors (VEGF, PDGF) and direct endothelial damage (increase in endothelial-derived microparticles). The sensitivity of CACs to environmental or demographic factors was further demonstrated in other human cohort studies where we show negative associations with exposure to the volatile organic compounds (benzene, acrolein) of polluted air or the exposure to volatile organic compounds. This work will further elaborate upon these findings and discuss how CACs are impacted quantitatively and qualitatively by multiple environmental influences.
The Role of Endothelial Progenitor Cells in Ambient Fine Particulate Matter (PM2.5)-Induced Atherosclerosis

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Exposure to ambient fine particulate matter (PM2.5) increases risks for cardiovascular disorders (CVD). However, the mechanisms and components responsible for the effects are poorly understood. We have shown that nickel (Ni) found on or in PM has been associated with an increased risk of mortality in human population studies and significant increases in vascular inflammation, reactive oxygen species generation, altered vasomotor tone, and potentiated atherosclerosis in murine exposures. To test our hypothesis that exposures decrease endothelial progenitor cell (EPC) count and impairs EPC function, which may ultimately have significant effects on various CVD such as atherosclerosis, experiments involving inhaled Ni nanoparticle exposures were performed in order to quantify bone marrow-resident EPCs using flow cytometry in C57BL/6 mice. Plasma levels of SDF-1α and VEGF were assessed by ELISA and EPC function was assayed in vitro in cultured bone marrow EPCs. Exposure to Ni nanoparticles significantly reduced bone marrow EPCs and changed EPC tube formation and chemotaxis. Plasma VEGF and SDF-1α differences were not statistically significant. In addition, a translational pilot study was conducted in female residents of Jinchang and Zhangye, China. Daily ambient and personal exposures to PM2.5 and 35 elements were measured in the two cities. A total of 60 healthy nonsmoking adult women residents were recruited for measurements of inflammation biomarkers. In addition, circulating endothelial progenitor cells (CEPCs) were also measured in 20 subjects. The ambient levels of PM2.5 were comparable between Jinchang and Zhangye (47.4 and 54.5µg/m3, respectively). However, the levels of nickel, copper, arsenic, and selenium in Jinchang were 82, 26, 12, and 6 fold higher than Zhangye, respectively. The levels of C-reactive protein, interleukin-6, and VEGF were significantly higher in Jinchang. Furthermore, all phenotypes of CEPCs were significantly lower in subjects recruited from Jinchang than those from Zhangye. These results suggest that specific metals, particularly Ni, may be important components responsible for PM2.5-induced cardiovascular effects and that the reduced capacity of endothelial repair may play a critical role.

Pulmonary Oxidative Stress and Impaired Growth Factor Signaling: Potential Mechanisms of Air Pollution-Induced Changes in Endothelial Progenitor Cell Homeostasis and Function

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Exposure to air pollution is associated with an increase in the risk for cardiovascular disease (CVD) and cardiovascular mortality. A hallmark of air pollution-induced cardiovascular toxicity is endothelial dysfunction. However, the mechanisms by which air pollution exposure triggers endothelial dysfunction and increases the risk for CVD are still not known. We showed that in mice, inhalation of particulates (concentrated ambient fine particulate matter (PM2.5), CAP) and volatiles (e.g., acrolein released during combustion of endogenous progenitor cells (EPCs)) by impairing their VEGF-mediated mobilization from the bone marrow. Our recent study also shows that, in mice, exposure to CAP induces an anti-proliferative, anti-angiogenic EPC phenotype, impairs EPC function, and reduces the ability of EPCs to promote vascular tissue repair. Interestingly, early changes in EPC levels due to CAP (or acrolein) exposure were accompanied by impaired vascular sensitivity to VEGF and insulin, growth factors that are known to regulate EPC mobilization and function. In line with this idea we found that pharmacological agents that improve insulin sensitivity (metformin or rosiglitazone) not only prevented CAP-induced vascular insensitivity but also restored EPC trafficking in CAP-exposed mice. Treatment with metformin or rosiglitazone also prevented CAP-induced inflammation and oxidative stress indicating that PM2.5 exposure decreases circulating EPC levels by a pro-inflammatory oxidative stress dependent mechanism. This is supported by our findings that overexpression of extracellular superoxide dismutase (ecSOD) in the lungs that attenuates CAP-induced pulmonary stress, also protects against CAP-induced vascular inflammation, vascular resistance to insulin and VEGF, and EPC depletion and dysfunction. These findings, suggest that upon PM2.5 inhalation, pulmonary generation of superoxide induces vascular inflammation and impairs vascular signaling that compromises morphogenesis of EPCs. The CAP-induced decrease in the ability of EPCs to maintain a healthy endothelium and to promote angiogenesis could result in endothelial injury, which could heighten the CVD risk.

Complex CNS In Vitro Models for Safety Testing: The More Complex the Better?

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Neurotoxicity is of complex nature and rarely predicted by the standard battery of in vitro safety tests applied in industry for early drug development. In addition, during the last years targets and therapeutic modalities got increasingly complex and sometimes for example large molecules drug candidates do not cross-react with any other species than human. This triggers a strong need for more complex cellular systems to be applied for safety testing. The central and peripheral nervous system is highly complex. Standard 2D systems do not necessarily enable the interplay of different cell types like neurons and glia cells crucial for physiological relevance of the model. Furthermore, as the accessibility of the CNS and PNS is limited due to implemented barrier systems the CNS and PNS are a challenge to model in vitro. The nervous system is a complex, dynamic organ. It is not only involved in the control of movement, the generation of thoughts, and feelings, but also has an important role in the control of the body's metabolic processes and its response to stress. It is therefore not surprising that the nervous system is one of the first systems to be affected by disorders such as stroke, Parkinson's disease, Alzheimer's disease, and multiple sclerosis. The nervous system is also one of the first systems to be affected by disorders such as stroke, Parkinson's disease, Alzheimer's disease, and multiple sclerosis. The nervous system is also one of the first systems to be affected by disorders such as stroke, Parkinson's disease, Alzheimer's disease, and multiple sclerosis. The nervous system is also one of the first systems to be affected by disorders such as stroke, Parkinson's disease, Alzheimer's disease, and multiple sclerosis.
tems as the blood-brain or blood-retina barriers, understanding compound disposition in those compartments is crucial for safety testing. These features cannot be recapitulated in a 2D system and 3D models or microphysiological systems would be of great value to mimic human physiology of the CNS or PNS to a very high degree. Are such systems already set up and ready for their use in drug development to test safety (and possibly efficacy) early on in a human relevant model? The need as well as the pros and cons of existing complex neural systems which are currently evaluated in-house for safety testing will be presented and the vision of their application will be addressed during the presentation. In addition, the existing and possible future requirements that will lead to a general use of these approaches for drug development will be discussed.

**3215 A Human BBB Microphysiological System for the Study of Neurotoxicity**


The human neurovascular unit (NVU) is a dynamic and highly organized structure that regulates biotransportation in the central nervous system (CNS). It consists of highly specialized endothelial cells (EC) that form the microvascular network, pericytes (PC) that wrap around the vessels, the basal lamina, and the end-feet of astrocytes extending to the vasculature. Together, they form a blood-brain barrier (BBB). While the BBB is exceptional at preventing foreign substances entering the CNS, there are compounds that can cross the barrier and cause neurotoxicity, including those with a high logP (low hydrophilicity that normally gives poor absorption and transportation) and those that are not cleared by P-glycoprotein and other efflux pumps. While many versions of a 2D BBB have been created, these fail to replicate the complex structure of the BBB and thus its unique capabilities. We have therefore developed an in vitro model of the human BBB with perfused vasculature, that allows for studies of BBB permeability and the pathological changes associated with inappropriate access of toxic drugs to the CNS. The platform is based on our human vascularized micro-organ (VMO) system where tissues are supported by nutrients delivered through perfused, living microvessels. Preliminary results demonstrate that the EC in the model display BBB characteristics, including expression of diagnostic transporters and junction molecules, and low overall permeability. We believe this model will prove to be an invaluable tool for testing the potential neurotoxicity of drugs in a human setting.

**3216 A Complex Human 3D Neural Cell System to Study Developmental Neurotoxicity**

H. T. Hogberg. Johns Hopkins University, Baltimore, MD.

Human in vitro models of brain neurophysiology are needed to investigate molecular and cellular mechanisms associated with neurological disorders and neurotoxicity. We have developed a reproducible iPSC-derived human 3D neural cell system, comprised of differentiated neurons (glutamatergic, dopaminergic and GABAergic neurons) and glial cells (astrocytes and oligodendrocytes). In this study we evaluated the developmental neurotoxicity effects of rotenone using high-content approaches, such as transcriptomics, metabolomics, immunohistochemistry and fluorescence assays. The data revealed that rotenone induced increased reactive oxygen species production (CellRos assay), and reduced mitochondrial dysfunction (MitoTracker assay). Furthermore, dopaminergic-neurons in the model seemed to be more sensitive to rotenone exposure than other neuronal cell types and glial cells (immunohistochemistry and gene expression data). Omics analysis showed changes in key pathways necessary for brain development, indicating rotenone as a developmental neurotoxicant and show a possible link between previously observed effects on neurite outgrowth and presently observed effects on Ca2+ reabsorption, synaptogenesis and PPAR pathway disruption. In addition, as our cell model reveals more than 40% overall myelination, something rarely seen in vitro so far, we evaluated the potential of chemicals to disturb myelination formation in our system. We selected four compounds shown to induce dys-myelination in animals (Cuprizone, BPA, Methylene, and TDCPP). Results showed that these chemicals also caused reductions in myelin formation in vitro at non-cytotoxic concentrations. This complex model has many advantages over simple monolayer cell cultures as it mimics better the in vivo situation. However, the throughput for toxicity testing is currently medium-low and the time it takes to differentiate the model is quite long and the costs are relatively high. Such a complex CNS model provides an excellent tool for future studies of neurological disorders such as multiple sclerosis, Parkinson’s disease as well as (developmental) neurotoxicity testing of drugs and chemicals.

**3217 Nerve-on-a-Chip Platform to Evaluate Peripheral Neuropathy with Clinically Relevant Metrics**


Electrophysiological and microstructural changes in peripheral and motor nerve tissue are arguably the most clinically relevant measures of pathology. These changes may manifest even before clinical symptoms arise, suggesting they may serve as more sensitive and physiologically-relevant metrics in an in vitro system. Our Nerve-on-a-Chip, an innovative preclinical model of both rat and human peripheral nerve tissue, enables electrophysiological and microstructural assessments in addition to cellular and molecular studies in a single, well-controlled preparation. This is the first in vitro model with the ability to perform nerve conduction tests and analyze histomorphometry, providing evaluation metrics of safety and efficacy that are analogous to those used clinically. We have successfully incorporated iPSC-derived sensory and motor neurons, astrocytes, and primary Schwann cells as well as primary rat dorsal root ganglion tissue to create a 3D in vitro Nerve-on-a-Chip which exhibits robust neurite outgrowth (up to 1 cm of growth) and myelination (up to 40% myelinated axons), and which has remained viable after two months, allowing us to perform more “chronic” and clinically relevant dosing regimens than a standard acute toxicity assay in vitro. Results on screening neurotoxic compounds using known chemotherapeutic and other agents with toxic mechanisms manifesting in changes to nerve conduction velocity, axon density, and degree of myelination have been able to predict clinical outcomes with historical compounds. We will present these results including dose responses to paclitaxel, bortezomib, vincristine, oxaliplatin, and others and compare to human clinical results. The ability to deliver high content, clinically-relevant data will enhance the field of preclinical neurotoxicology testing in efficiency, cost, and accuracy. In addition, I present a review of interaction with end users of MPS and organs-on-chips to provide insight on the relationship between model developer and biopharma. This discussion aims to better elucidate the place in which microphysiological systems fit within the drug development pipeline, both now and in the future, from the perspective of the MPS industry.

**3218 Retina-on-a-Chip: Merging Organoid and Organ-on-a-Chip Technology for Complex Multi-Layer Tissue Models**

P. Loskill. Fraunhofer Institute for Interfacial Engineering and Biotechnology, and Eberhard Karls University Tübingen, Tübingen, Germany. Sponsor: H. Hogberg

Retinal diseases such as age-related macular degeneration or retinitis pigmentosa are the leading cause of blindness. However, there is often no cure or treatment available, which is partly due to a lack of suitable model systems. Additionally, many substances induce retinal toxicity in the neuronal and/or the retina/blood-barrier component. To study retinal diseases and toxicities different model systems are available: i) in vivo animal-based models feature various similarities with the human retina but also significant differences e.g. not trichromatic or missing macula, ii) ex vivo models based on human retinal tissue extracted post mortem are problematic due to their limited availability and cultivability, iii) in vitro cell culture models mostly utilize cell lines and are not able to recapitulate the complex physiological structure and functionality of retinal tissue. Recent advances in stem cell biology and the possibility to generate 3D organ-like structures have opened up new possibilities for pharmaceutical and toxicological research. Yet, those so-called organoids are still limited by shortcomings such as proper functional maturation and the physiological interplay of retinal cells. The Organ-on-a-Chip (OoC) technology has the potential to address those limitations by creating physiological accurate in vitro models of human tissues in a microfluidic environment. However, it is extremely difficult to recreate the complex, stratified 3D architecture of the human retina featuring a large number of cell types within an OoC in its entirety. By merging OoC and organoid technologies, we have developed a microphysiological Retina-on-a-Chip, which successfully recreates the tissue structure and recapitulates physiological cell-cell interactions. The combination of the biological self-assembly mechanisms in organoids with the precise micro engineering of OoCs, enabled us to culture organoids containing the neuronal components of the retina and retinal pigmented epithelial cells in a vascularized environment and to observe a so-far unmatched functional interplay of the different cell types. The developed 3D retina chip is extremely versatile and applicable for drug development, toxicity screening and disease modeling.
Order from Chaos: Pattern Recognition in Challenging Human Health Datasets

J. Wambaugh. US EPA, Research Triangle Park, NC.

The science of complex systems has demonstrated that while some things about noisy, real-world data are unknowable, certain patterns and structures can emerge from the chaos. Given the increasing complexity of data related to assessing potential risk posed to human health by chemicals, this session is intended to incubate new methodologies for mining “Big Data” to inform functional human health outcomes. Driven by innovations in computational techniques, many problems that were once intractable can now be understood in terms of these recurring patterns. While the areas of research in this session are diverse, there is a surprising commonality about the challenges faced by researchers and the potential cross-domain applicability of the approaches used to solve the problems. The session begins with an exposomics-based approach to understanding the role that environmental chemical exposures may play in public health outcomes. The workshop continues by addressing metabolomics and novel, non-targeted analysis (NTA) of chemicals in environmental and biological media. NTA analysis generates thousands of chemical features per sample, and each sample contains information about the potential upstream chemical sources or pathways. Algorithms can identify unique feature signatures associated with sample groups, allowing understanding of sample content and history and development of source hypotheses. The session will then explore the world of in vitro testing where, among the hundreds of cell lines available, efforts are being made to identify a parsimonious few that explain as much phenotypic variability as possible. The final presenter is from a large, multi-national consulting firm. The presentation will examine prediction of human health outcomes using new analytics techniques to identify environmental toxin “Hot Spots” using Big Data. In all five presentations, identifying patterns in complex data to allow for more informed decision-making. Each presentation will consider: (1) What are the challenges of the system of study? (2) What aspects of that system are unknown or unknowable? (3) What patterns emerge from the complexity of the system? (4) What tools are available for identifying these patterns? (5) What are the human health implications for the patterns that can be recognized?

Informatics and Data Analytics to Support Exposome-Based Discovery for Public Health

J. Chung, and C. Patel. Harvard Medical School, Boston, MA. Sponsor: J. Wambaugh

Epidemiologic studies attempt to understand etiology through associations between environmental exposures and phenotype; from large genetics investigations, it is apparent that genetics explain a fraction of chronic diseases. Recent advances in measurement technologies create huge research opportunities to understand the true influence of environmental exposures in the exposomics era. However, the promise of high-throughput exposomics research is accompanied with increasing difficulty to decipher the subtle relationships due to complex data, including: dense correlational structure, low association sizes, mixture exposures, high dimensionality, missing values (especially an issue for chemical measurements), and high spatial, temporal, and individual variability. In addition, there are analytical challenges stemming from a lack of statistical power and reproducibility of findings. In contrast to the previous big data transparency workshop, here we focus on practical topics in exposomics-based research. Specifically, we will discuss analytical solutions to some of the data challenges, such as machine learning methods to understand patterns and structure in data, and emerging ways to systematically detect the associations between exposures and disease. We will also discuss briefly the need for setting up data standards and infrastructure for future exposomic studies.

Finding Patterns within Complex Metabolomics Datasets

B. van Ravenzwaay. BASF SE, Ludwigshafen, Germany.

The MetaMapTox data base contains the metabolome profiles of more than 800 compounds, obtained from rat blood samples in short-term studies. A prediction of toxicity of new chemicals is obtained by comparison with (1) > 100 sets of fixed metabolite regulations associated with a particular form of toxicity/mode of action, and (2) a ranking of the overall metabolome of the compound under investigation with all other available profiles. The high accuracy of prediction (> 80%) we have achieved is related to two features: the size of the data base, and the unique rigor of control of the experimental set-up. Recommendations from our 15 years of experience should be applicable to any of the ‘omics technologies. Control and understanding of variability, arising from the biological assay, sampling and storing procedures as well as from the measurement itself, is essential. At the start of a large-scale, multiple year-project extensive investigation in control variability is important. Using of reference (positive control) substances the signal/noise ratio should be determined. Once a satisfactory ratio has been obtained all procedures should be documented in standard operating procedures and followed meticulously during the rest of the project. It is strongly recommended to regularly perform exact controls and confirmatory controls to obtain information on the variability of positive responses. Control data should be regularly checked for any shifts and analyzed to obtain information concerning normality. Large sets of control data can also be used to obtain an historical-range for individual parameter variability. If possible, data should be analyzed by multiple procedures and conclusions should be drawn based on a combined assessment. We recommend to perform such final evaluations in a team of experts, not unlike peer review processes in histopathology.

Identifying Chemical Signatures of Manufacturing and Recycling in Household Products


This presentation will describe the challenges inherent in understanding high-resolution mass spectrometry (HRMS) data generated in non-targeted (NTA) analyses of the chemical constituents of household products. These approaches can help identify pathways that can be captured in the bands of molecular features (and thus chemicals) in a single analytical run without any a priori knowledge of the compounds present. These studies are being used by US EPA to screen consumer products articles of commerce for chemical content. These NTA screens generate large numbers of chemical features that are understood in part through comparison against large, newly compiled databases of chemical ingredients and weight fractions in household products. However, not all chemicals can be identified in these databases. Consumer product ingredient databases have therefore served as training sets for machine learning tools capable of predicting the functional use of chemicals in consumer products based upon structure alone. These tools prioritize chemicals for further study and identify novel compounds. Across many media samples of a given type, analysis of these features can uncover co-occurring groups of chemicals (“signatures”) that may be indicative of unique exposure sources. In consumer products, signatures could be associated with intentional (functional) addition, manufacturing process, or contamination. While unsupervised clustering and co-clustering methods are useful in elucidating local patterns, there are challenges in applying them to HRMS data. True variability in chemical content across samples, reproducibility in analytical results, and uncertainly in mapping of molecular features to chemicals all contribute to noise that imparts a large degree of complexity (and thus computational burden) to the problem. Where sample metadata are available, supervised or semi-supervised approaches can be used to further focus the analysis. Case studies of unsupervised and supervised approaches will be presented and challenges discussed. Development of standard methods and tools for identifying chemical signatures can improve identification, prioritization, and mitigation of chemicals in consumer products. This abstract does not necessarily reflect US EPA policy.

Parsimonious Selection of Cell Lines to Reproduce Phenotypic Variability

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High-throughput transcriptomics can cost-effectively screen thousands of chemicals to identify changes in 3,000 to 20,000 transcripts in human cell lines. However, it is known that screening two cell lines over the same set of chemicals may give variable responses. If one cell type is decided, how can we efficiently pick the next cell type to screen where the responses will be complimentary and not entirely redundant? We hypothesize that a data-driven approach can choose a limited number of cell types that will reflect variability of chemicals may give variable responses. If one cell type is decided, how can we efficiently pick the next cell type to screen where the responses will be complimentary and not entirely redundant? We hypothesize that a data-driven approach can choose a limited number of cell types that will reflect a majority of the relevant biology for toxicity testing. Specifically, we have evaluated the genetic diversity in baseline gene expression data over >1000 human biological samples utilizing inter-cell differences in a “content maximization” approach. Maximizing phenotypic variability included choosing cell lines that displayed diversity in the most influential factor explaining the diversity, which was organ lineage. Surprisingly, organ lineage was identified as the most influential factor using either the baseline gene expression data, or a dataset of differential toxicity responses from 600 chemicals. This approach provides a data-driven way to examine cell line diversity to ensure adequate coverage of biological space, which will impact the way we choose cell lines for high-throughput approaches.
Identifying Environmental Toxin “Hot Spots” Using Health Care Data

M. Mardorff, Deloitte Consulting, Harrisburg, PA. Sponsor: J. Wambaugh

Deloitte is a multi-national professional services firm with more than 71,000 professionals in the US and more than 270,000 worldwide. At Deloitte there is interest in mining Big Data to be predictive of functional human health outcomes to support better and more timely responses by health and human services agencies. Vast amounts of data are characterized by Medicaid claims/encounters, All Pay Claims databases, and most recently statewide Health Information Exchanges (HIE). The data have the potential to be used to identify, and potentially address, “Hot Spots” (these could be geographic, demographic, etc.) due to adverse effects induced by environmental toxins. Data sets that are available to states via HIE is especially interesting: with the correct machine learning model and process we could support the scanning of data flowing through an HIE and identification of hotspots in near real time. In a different but related context, we have recently used this approach and these same data sets to identify opioid abuse hotspots along with enabling prescribers.

Risk Assessment of Consumer Products and Articles: Critical Considerations and Case Studies for Characterizing and Quantifying Consumer-Relevant Exposures to Chemicals and Nanomaterials

S. Felter, Procter & Gamble Company, Mason, OH.

Increasingly, toxicologists are challenged to do risk assessments for consumer products for which the determination of relevant exposure is not straightforward. Quantification of potential exposure from many types of existing and emerging consumer products (e.g., textiles, diaper, diaper printed products) is still an emerging science and the application of nanomaterials introduces even further challenges. Quantifying exposure that mimics actual and foreseeable consumer use associated with a range of consumer products can require advanced sampling approaches and analytical capability. This raises the question of how we should define what is “reasonably conservative” versus “not relevant” when developing methods. Given the limitations/absence of these capabilities and/or accepted methods, overly conservative methods and assumptions are often used that are not relevant to consumer exposures or represent extreme worst-case use scenarios. The session will provide examples of frameworks and sampling and analytical methods that have been developed to determine exposures from products and articles that are relevant for actual consumer use scenarios. Case studies include the estimation of chemical migration and relevant consumer exposure estimates to engineered nanomaterials along the product value chain, release of silver from nanotechnology-based children’s products, and the potential for migration of any constituents above a TTC-based threshold from a disposable diaper. Factors that are important to consider when developing extraction methods for mimicking consumer use scenarios, including relevant solvents, will be discussed. These data can then be used in robust risk assessments leading to informed decisions on safety decisions that in such products under normal usage conditions and improve product safety and risk communication to the public. This session will also consider what exposure data are needed by regulators associated with the new Frank R. Lautenberg Chemical Safety for the 21st Century Act and how end uses of a chemical are considered when estimating the potential for consumer exposure.

Introduction: Relevance of Exposure Data for Toxicological Research and Risk Assessment of Chemicals and Emerging (Nano) Materials in Consumer Products

T. A. Thomas, Consumer Product Safety Commission, Rockville, MD.

Assessing potential health risks from chemicals and emerging materials incorporated into consumer products/articles (e.g., clothes, toys, furniture, hygiene products) requires robust data from a number of disciplines including toxicology, exposure science and risk assessment. The releases of chemicals of interest from products and subsequent are influenced by a number of factors including product use patterns and exposure science plays an important role in developing methods to adequately characterize and quantify consumer exposures. The toxicology community must produce data that is relevant for these real-world consumer exposures. Risk assessors must also engage with these communities to ensure the development of exposure and toxicity data and that this information is appropriately incorporated in risk assessments and the results communicated to stakeholders. As emerging materials, such as nanomaterials continue to be commercialized, their potential health impacts will be assessed along with other chemicals. In 2018, the Society of Toxicology took an important step forward in embracing exposure science by developing an exposure assessment specialty section. This workshop will explore the relationship between exposure assessment and the relevance of exposure data for toxicity research and risk assessment for traditional and emerging chemicals and materials, and how information for these various classes of compounds can be utilized to develop more robust risk assessments for a range of chemicals in consumer products. Case studies will be presented that highlight these concepts.

Understanding the Changing Exposure and Toxicity Profile of Engineered Nanomaterials from Production to Application

A. Erdely, NIOSH, Morgantown, WV.

Engineered nanomaterials, because of their electrical, chemical, and thermal properties, are being incorporated into existing, everyday products, with broad applications to medicine, electronics, composites, and construction. From smart phones, to water purification, to cosmetics, to thermoplastics (e.g., toys, containers), human exposure to engineered nanomaterials and their applications is inevitable. It is critical that interpretations of potential toxicity along the product value chain be exposure-informed as TSCA requires a risk evaluation at all points along the life cycle of a chemical. Properly understanding and developing risk profiles for workers and end-users are necessary to prevent unintended health consequences. Engineered nanomaterial research initially focused on as-produced (pristine) material with little attention to downstream applications. Given the broad applications of nanomaterials into existing and emerging technologies, a more expansive characterization of exposure was needed to understand potential health risks. To accomplish this goal, a multidisciplinary team with private sector partners was needed. This work will describe a comprehensive case study evaluating carbon nanotubes, which represent a highly visible engineered nanomaterial due to the significant toxicity observed following in vivo evaluations. The work evaluates the changing toxicity and exposure characteristics along the product value chain from the production of the as-produced material, to post-production modification, to matrix (or product) incorporation. The results clearly indicate that exposure and toxicity, and thus potential risks to workers and end users can be quite different from product to product. Understanding the changing exposure profile of engineered nanomaterials along the product value chain is critical for determining potential human health risks and overcoming risk-driven concerns that are potential barriers to increased commercialization. The case study also provides a framework and recommendations for evaluating other materials and scenarios, develops reproducible methods, and highlights new, or alterations to existing, methodology to characterize exposure and toxicity at different points along the product value chain. The comprehensive approach of combining toxicity and exposure assessment is necessary to provide direct inference to potential consumer risks.

Estimating the Release of, and Exposure to, Silver from Nanotechnology-Based Consumer Products for Children

M. E. Vance. University of Colorado Boulder, Boulder, CO. Sponsor: S. Felter

Silver nanoparticles (nanosilver) are gaining significant attention in the academic and regulatory communities, not only because of their antimicrobial effects and subsequent product applications, but also because of their potential health and environmental impacts. Although some human health effects of silver and silver nanoparticles have been reported, realistic exposure levels from the use of consumer products must be estimated to inform toxicity studies. The objective of this work was to characterize the release and potential exposure to silver and silver-containing particles during the normal use of silver nanotechnology consumer products for children. We developed a framework to assess (1) whether products contained silver, (2) whether silver in products was in nanoparticulate form, (3) whether products might release silver under realistic usage scenarios, and (4) in what form and concentration silver releases are most likely to take place. To put this framework to use, we compiled an inventory of 82 children’s consumer products that claim to contain nanosilver and selected 13 products for presence of silver and its release into liquid media, into air, and onto skin. All products contained some form of silver, but silver-containing particles were observed in only four products, with sizes ranging from nanoscale up to 10 µm in size. Silver leached preferentially into synthetic biological media with higher chloride concentrations, such as sweat and urine. We determined that levels of silver to which children would be exposed during normal use of these products are likely to be low, and bioavailable silver is expected to be in ionic rather than particulate form. This framework may be used to assess exposure from other increasingly popular nanotechnology-enabled consumer products.
Modern disposable diapers are composed of large polymeric materials that are not bioavailable. In some cases, there might be other aspects of a diaper such as a lotion applied to the topsheet, which are intended to transfer to the skin to provide skin health benefits. But what about the diaper itself? Will smaller molecular weight constituents (eg, fiber finishes) transfer to the skin? What about constituents present in the core or other part of the diaper that is not in direct skin contact? While diapers are associated with a long-history of safe use, how should this be substantiated prior to marketing? One approach is to conduct paper-based safety assessments based on supplier disclosures of starting materials used in making diaper components and applying conservative assumptions about how much might actually come into contact with the infant. While this is generally considered to be quite conservative, it does not accurately portray what an infant will be exposed to during normal diaper wear. In many cases, the actual exposure to substances used in the making of a diaper will be negligible. However, it is also recognized that analytes might be detected that were not in the supplier disclosures. To develop a more rigorous approach to the safety evaluation of diapers, we developed methods that involve subjecting a diaper to conditions reflective of consumer use, including use of physiologically-relevant amounts of solvent (surrogate for urine), and applying an amount of pressure to the diaper relevant to that of an infant sitting. This is followed by analytical analysis of the small amount of liquid (“rewet”) that can be collected, which would include any low-level constituents in the diaper that could be solubilized by urine and brought back to the surface in contact with the infant. This is in contrast to targeted analyses focused on predetermined substances of interest. The Threshold of Toxicological Concern (TTC) is used to establish a level above which a compound would need to be identified and below which safety is not a concern. The final safety assessment is conducted on all constituents detected above TTC. This talk will present a case study, demonstrating the quantitative safety assessment of diaper components using methodology based on physiologically relevant exposure conditions.

On June 22, 2016, the Frank R. Lautenberg Chemical Safety for the 21st Century Act was signed into law thereby amending the Toxic Substances Control Act (TSCA). This new legislation is the first update to TSCA in 40 years. Consumer products and articles are significant sources of indoor chemical exposures. Exposure estimates can be developed using measured exposure data, modeling approaches, or a combination of both. The US EPA’s Office of Pollution Prevention and Toxics evaluates exposures to new and existing chemicals, including nanomaterials, and has augmented their suite of exposure estimation tools with updates to the Consumer Exposure Model (CEM), the Indoor Environmental Concentrations in Buildings with Conditioned and Unconditioned zones (IECCU) model, and the development of indoor exposure testing protocols to experimentally determine exposure parameters. The experimental results can be used on their own, or in combination with exposure models to quantify consumer exposures. Updates to CEM include improved use, and functional use category definitions through the OECD can be used to inform development of consumer exposure scenarios. This presentation will provide an overview of the interrelatedness of consumer exposure models, available empirical data, indoor exposure testing protocols, and use descriptors in estimating consumer exposures. The views expressed in this abstract are those of the authors and do not represent Agency policy or endorsement.

Millions of Americans use e-cigarettes, even as rates of smoking combustible cigarettes continue to decrease among young adults. Recent survey data show that e-cigarette use is greater in youth (under age 18) compared with adults (age 18 and older); among adults, e-cigarette use is generally greater among young adults and decreases with increasing age. In May 2016, the US Food and Drug Administration (US FDA) issued a rule which extends its regulatory authority to all products that meet the “substance use disorder” definition of tobacco product, including e-cigarettes. To gain insight into the risks and benefits of e-cigarettes, the US FDA’s Center for Tobacco Products, by congressional mandate, requested the National Academies of Sciences, Engineering and Medicine (NASEM) to convene a committee of experts to conduct a review of the evidence about the public health consequences of e-cigarette use. This discussion led by Dr. Vorhees and Dr. Talpos on the evidence for increased vulnerability in adolescents compared with adults and the extent to which proposed neurocircuit targets and biological markers are unique to teenage vulnerability for dependence. They will also evaluate whether approaches are generalizable to toxicity testing to screen for effects of chemical and drugs that may increase susceptibility of teenagers to substance use disorders (SUD). Participants will discuss implications of unique patterns of behavioral, neurochemical, and other biomarker changes in adolescents for public health. Ms. Kwan, a graduate student in Public Policy and Public Administration at George Washington University, who has been involved with research on age of initiation of nicotine on public health outcomes, will briefly introduce the topic and frame the overarching questions for the session. Dr. Eaton will set the stage by presenting recently published (2018) results from one of the most comprehensive studies by the National Academies that he chaired on human health effects of e-cigarettes, including youth initiation. Dr. Levin will begin with a brief introduction on neurochemical and anatomical pathways of addiction followed by presentation of ongoing research on behavioral, anatomic, and signaling pathway markers of nicotine dependence in adolescent and adult rats. Dr. Dow-Edwards will compare and contrast the effects of delta-9-tetrahydrocannabinol (d9THC), the chemical responsible for most of marijuana’s psychological effects, on adolescent brain-behavior relationships with emphasis on male-female differences in alterations of neural circuits mediating these relationships. Dr. Andersen will present a state-of-the-art translational approach showing how a dopamine receptor mediated “switch” underlies age-related periods of drug-induced “protection” or drug-induced vulnerability associated with addiction with the ultimate goal of developing targets that can be used in teenagers to reduce addiction. The workshop will end in a 40-minute panel discussion led by Dr. Vorhees and Dr. Talpos on the evidence for increased susceptibility and identification of data gaps that will encourage cross-fertilization of ideas for development of novel screens for the potential of chemicals and drugs to increase susceptibility of teenagers to SUD. This session introduces an important new scientific area to SOT; namely, the vulnerability of the adolescent brain to chemical/drug exposure. It will be of interest to a broad audience, including those interested in neurotoxicology, public health, clinical and translational toxicology, drug discovery toxicology, and social implications of this science.

Human variability is an important consideration in toxicology and risk assessment. Significant advances have been made to address differences between the adult and fetus/children or the elderly. In contrast, adolescent teenagers are generally considered to be smaller adults when considering the toxic effects of drugs and chemicals. With the recent legalization of recreational marijuana in California, it is generally considered to be quite conservative, it does not accurately portray what an infant will be exposed to during normal diaper wear. In many cases, the actual exposure to substances used in the making of a diaper will be negligible. However, it is also recognized that analytes might be detected that were not in the supplier disclosures. To develop a more rigorous approach to the safety evaluation of diapers, we developed methods that involve subjecting a diaper to conditions reflective of consumer use, including use of physiologically-relevant amounts of solvent (surrogate for urine), and applying an amount of pressure to the diaper relevant to that of an infant sitting. This is followed by analytical analysis of the small amount of liquid (“rewet”) that can be collected, which would include any low-level constituents in the diaper that could be solubilized by urine and brought back to the surface in contact with the infant. This is in contrast to targeted analyses focused on predetermined substances of interest. The Threshold of Toxicological Concern (TTC) is used to establish a level above which a compound would need to be identified and below which safety is not a concern. The final safety assessment is conducted on all constituents detected above TTC. This talk will present a case study, demonstrating the quantitative safety assessment of diaper components using methodology based on physiologically relevant exposure conditions.

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Drug addiction is a particularly nefarious form of neurotoxicity, inasmuch as one of the principal neurotoxic effects is to cause those exposed to dose themselves with more of the toxic chemical. Adolescence is an especially vulnerable time for the initiation of drug addiction. Neural systems involved with addiction are still developing during that period. Nicotine is the principal neuroactive chemical in tobacco and is central to the addictiveness of tobacco. The great majority of people who become addicted to tobacco start during adolescence, when the brain is still undergoing important phases of development. Does adolescent onset potentiate addiction? This is difficult to determine in humans since there are a number of different causative factors underlying the substantial adolescent onset drug use problem. The same genetic/environmental conditions that predispose individuals to tenacious addiction also could induce them to start using early; drug use during adolescence could shape brain development around the addictive behavioral pattern; or the increased plasticity of adolescence could be a more fertile ground for the growth of addiction. With animal models we can determine the causative nature of adolescent vs. adult-onset nicotine self-administration with random assignment to drug-taking groups that is not possible in humans. We have found in a series of studies that male and female rats that are given access to nicotine self-administration during adolescence self-administer significantly more nicotine than rats first given access in adulthood. Male adolescent rats have higher rates of nicotine self-administration than female adolescent rats; female rats that start nicotine self-administration in adolescence have a more persistent elevation of self-administration as they mature into adulthood. Adolescent-onset nicotine self-administration also causes long-term impairment in regulation of nicotine intake. In other studies, we have shown long-term adverse behavioral effects of gestational nicotine. Like many chemicals nicotine has differential neurobehavioral toxicity depending not only on dose but also age.

**Sex-Dependent Effects of Delta-9-Tetrahydrocannabinol on Adolescent Brain-Behavior Relationships**

D. Dow-Edwards. SUNY Downstate Medical Center, Brooklyn, NY. Sponsor: A. Li

With increasing legalization of marijuana, the public hears little about the toxic effects of the drug. Marijuana can interfere with experience-dependent plasticity, the foundation of cortical neural circuitry development during adolescence which is largely completed by adulthood. During adolescence, delta-9-tetrahydrocannabinol (THC) administration alters brain-behavior relationships differently within circuits subserving affect, reward and cognition. These alterations exhibit period-specific and sex-specific vulnerabilities. For example, THC administration during the prepubescent period produces significantly greater effects on affective behavior than later exposure and since males and females undergo puberty at different ages, the age of maximal effect is different in males and females. We also have data showing that males and females respond differently to early stress such that during mid-adolescence, females show alterations in cannabinoid receptors in a specific constellation of brain regions while males show a different constellation of effects. Also, studies have demonstrated that adolescent exposure to THC produces permanent and even transgenerational effects on reward circuits by increasing the consumption of drugs of abuse while similar exposures to the adult do not. Emphasis will be placed on marijuana toxicities determined in relevant animal models in comparison with available human data in this relatively understudied population. The dose-response nature of the relationship between marijuana and neurotoxicity will be highlighted to emphasize the message that consuming less marijuana, less frequently and beginning at an older age will reduce the overall impact of smoking marijuana during adolescence on adult brain function.

**Panel Discussion with Drs. Charles Vorhees (Cincinnati Children’s Hospital) and John Talpos (US FDA/NCTR)**

C. V. Vorhees1, and J. C. Talpos2. 1Cincinnati Children’s Hospital Medical Center, Cincinnati, OH; and 2US FDA/NCTR, Jefferson, AR.

Two discussants will preview the presentations prior to the meeting so that there will be vigorous productive discussion on the evidence for increased susceptibility and identification of data gaps. This abstract highlights some of the questions that break down some of the issues that need to be discussed in order to address the overarching question of whether there are biological markers unique to adolescent vulnerability to dependence toxicity. There are human data indicating that drug initiation during adolescence poses a greater risk for addiction and long-term effects than when drug use starts later in life. To what extent do the human data allow distinction between whether the increased risk is due to biological immaturity, underlying biological susceptibility to dependence, or psychosocial and environmental factors? There are numerous studies testing the effects of drugs on adolescent rodents but every lab seems to define the adolescent period differently. What is the definition of adolescence in rodents and humans, how is it differentiated from puberty, and is it the same in males vs. females? Dopamine and dopamine receptors play key roles in addiction and dopamine projections undergo synaptic pruning during adolescence: what is the role of these processes in the vulnerability of the adolescent brain? What evidence indicates that adolescent exposure is unique and qualitatively or and quantitatively different from the same exposure to adults? To what extent does this evidence support specific behavioral, neurochemical or other endpoints as unique biomarkers of adolescent vulnerability to dependence toxicity. Does the evidence suggest that these biomarkers depend on the specific drug or are there consistent patterns of change across different classes of drugs? What, if anything, can science practically contribute as recommendations for clinical practice or drug development toxicity evaluations to reduce or prevent addiction/drug abuse in teenagers? Dr. Vorhees studies the developmental effect of chemicals using neurobehavioral and genetic animal models of CNS disorders. Dr. Talpos worked in a drug-discovery setting developing pro-cognitive treatments for psychiatric and neurodegenerative disorders prior to joining US FDA where he runs the nonhuman primate translational cognitive program.
Influenza A viruses (IAV) cause severe disease in humans and are a significant public health problem. Contemporary epidemiology studies show that dioxin and PCB levels are associated with increased incidence and severity of respiratory tract infections and poorer vaccine responses in infants and children. These compounds bind the aryl hydrocarbon receptor (AhR), an environment sensing transcriptional regulator. In addition to dioxins and PCBs, humans are exposed to other AhR ligands regularly, further emphasizing the need to elucidate how AhR signaling alters host immune responses during viral infection. The mechanisms by which AhR signaling modifies antiviral immune responses are poorly understood. One cell type critical in mediating antiviral host responses is the dendritic cell (DC). DCs are highly specialized antigen presenting cells and bridge innate and adaptive immune responses. DC function is perturbed following exposure to AhR ligands, and this is directly tied to poorer outcomes following in vivo IAV infection. Using a combination of FACS and RNA-Seq, we show that AhR activation during IAV causes significant changes to the DC transcriptome, including genes associated with viral uptake and processing. A major virulence factor of IAV, the non-structural protein 1 (NS1), plays a role in influencing DC gene expression and function. Utilizing state-of-the-art fluorescence expressing IAV, we show that AhR activation increases IAV levels within infected DCs, suggesting that AhR signaling may affect viral responses within DCs. IAV lacking NS1 protein (∆NS1) is replication defective. Infection of mice with ∆NS1 IAV combined with AhR activation did not alter morbidity or mortality; however, serum anti-IAV antibody levels were significantly reduced. These data suggest that AhR activation may alter the ability of DCs to stymie IAV NS1 function. Thus, AhR activation modulates intracellular antiviral defense systems in DCs, providing new mechanistic insight to explain why exposure to environmental AhR ligands is associated with greater morbidity and decreased adaptive immune responses upon primary viral infection.

**Mechanism(s) of Th2 Polarization in Mouse CD4+ T Cells Exposed to the Food Preservative, tBHQ**

Michigan State University, East Lansing, MI.

The United States has seen a dramatic increase in food allergy prevalence over recent decades. Though the etiology of this disorder is multifactorial, environmental factors, including certain food additives, have been implicated. tert-Butylhydroquinone (tBHQ) is a food preservative known to activate the nuclear factor erythroid 2-like 2 (Nrf2) transcription factor to induce antioxidant gene expression. Data from our lab suggest a previously unknown immunomodulatory role of Nrf2, where tBHQ activation induced Nrf2-dependent skewing of CD4+ T cells towards a Th2 (allergic) phenotype. Polarization resulted in increased production of Th2 cytokines (IL-4, IL-5, and IL-13) and DNA binding of the master regulator of Th2 differentiation, GATA3. To date, the molecular mechanism(s) responsible for increased GATA3 activity and subsequent Th2 cytokine protein production are unknown and elucidation of this mechanism(s) is the goal of this study. Primary mouse CD4+ T cells (WT and Nrf2-null) were treated with tBHQ and GATA3 activity was analyzed. Additionally, Nrf2 binding to putative AREs identified within an intron and the promoter of GATA3 was demonstrated in D10 cells. The results of these experiments suggest that GATA3 is regulated at the transcriptional level by Nrf2. First, GATA3 gene expression and protein production increased after tBHQ treatment in WT, but not Nrf2-null, mice. Second, Nrf2 binding to putative AREs within an intron and the promoter of GATA3 was demonstrated in D10 cells. Finally, the functionality of these putative AREs was established by luciferase assay. These data demonstrate Nrf2-dependent upregulation of GATA3 in Th2 polarization of CD4+ T cells, which could have potential implications in Th2 mediated disorders such as food allergy. This study was supported by NIH grants ES024966 and ES725530.

**Anti-Metallothionein Management of Chronic Inflammatory Disease**

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Metallothionein (MT) is upregulated as a consequence of cell and tissue encounters with a variety of stressful conditions. Basal MT levels are responsible for management of essential divalent heavy metal cations and redox metabolism, with the exception of Cu and Zn, which are primarily maintained in healthy individuals and serve as a target for reactive oxygen species. In light of these essential housekeeping roles, research has largely focused on the intracellular pool of MT and how stress can disturb these housekeeping roles. In that context, we have found that MT manages intracellular free Zn in a range that can modify intracellular signal transduction in CD4+ T cells. Although it lacks a signal peptide for export, a less recognized extracellular pool of MT can be found in cases of stressor exposure. Its release is not blocked by Brefeldin A or monensin. Unlike some heat shock proteins, MT does not appear to be released via the exosome pathway of unconventional protein secretion. This extracellular pool influences the progression of humoral responses to T-dependent antigen challenge. As a consequence of its sequence similarities to closely linked chemokine genes, we have explored the ability of extracellular MT to act both as a chemotactic factor itself, and to interfere with the cellular responses to other chemokines. MT is able to initiate chemotaxis of a variety of cell types, including monocytes, macrophages, T lymphocytes, spleenocytes, and cloned mammary tumor cells. This movement can be blocked with either cholera toxin or pertussis toxin, suggesting that MT initiates chemotaxis via interaction(s) with G protein-coupled receptors (GPCR). Inhibitors of signaling elements (e.g. Arp 2/3 and PLC) downstream of GPCRs can block MT-mediated chemotaxis. Pre-incubation of Jurkat T cells with MT blocks the subsequent response to CXCL12 (SDF-1α), a known ligand for CXCR4 and SDF-1α can block MT-mediated chemotaxis. Moreover, AMD3100, a known antagonist of SDF-1α binding to CXCR4 can also block MT-mediated chemotaxis. A monoclonal antibody to MT, clone UC1MT, has been shown to interfere with MT-mediated chemotaxis, suggesting that UC1MT might be able to diminish inflammation in vivo when stress-induced MT is released from cells to establish a pro-inflammatory positive feedback loop leading to inflammatory disease. Treatment in several mouse models of inflammatory bowel disease (IBD) with UC1MT, including an adoptive T cell transfer model, has been found to significantly diminish the progression of that disease.
Gastrointestinal disorders are highly prevalent among veterans who suffer from Gulf War Illness (GWI), a series of pathological events that plague the veterans of the first Gulf War (1990-1991). Most of the associated pathologies have been ascribed to chemical exposures like Pyridostigmine Bromide (PB) and insecticide Permethrin, diethyltoluamide (DEET) and organophosphates. Symptoms include fatigue, pain constipation, and diarrhea similar to inflammatory bowel disease (IBS). Our previous studies have linked some of these symptoms to an altered gut microbiome which results in inflammation in the gut. The present study uses a mouse model of GWI and investigates the role of a unique virome and phageome signature in influencing the microbiome. The results showed that the alpha diversity that depicts virome abundance decreased significantly in GW-chimical treatment while antibiotic treatment restored their diversity. Further, our study for the first time has successfully tracked a phageome signature in a GWI rodent model. The results showed that Bacteriophages of family Siphoviridae, known to attack probiotic bacteria increased by 40% compared to the control groups, while family Podoviridae was reduced significantly. The results associated strongly with alteration in the microbiome with a loss in Bacteroidetes and increase in Firmicutes phyla, that has been reported in other bowel disorders such as IBS and IBD. Furthermore, mice treated with war theatre chemicals (PB+PER) that had decreased viral diversity and suppressed Siphoviridae phage showed an increase in claudin-2 expression and a reduction in occludin tight junction protein. The above group also had an increase in mRNA expression of inflammatory markers CD68 and IL-1β. Antibacterials restored viral diversity while treatment with antiviral drugs caused a marked increase in inflammatory markers compared to antibiotic-treated mice. Taken together, these results indicate that bacteriophages might greatly influence the health of the gastrointestinal tract via regulating the microbiome and paves way for a possible therapeutic strategy.

Inorganic arsenic (iAs) is a potent carcinogen and immunotoxicant, which poses a risk to millions of people worldwide through the consumption of contaminated water and food. iAs exposure in adults and children has been correlated with increased gastrointestinal and respiratory tract infections, including tuberculosis (TB) and influenza, often in a sex-dependent manner. Environmental exposures are thought to contribute to the persistent global burden of vaccine preventable infectious diseases. However, it is unclear what role the sex-specific mechanisms have played in these phenomena. Studies in animal models and human populations suggest the aryl hydrocarbon receptor (AhR) provides a link between environmental exposures and immune responses later in life, but little is known about the cellular and molecular mechanisms driving these durable changes. Recently, we showed that developmental AhR activation, by maternal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, the prototype AhR agonist), impairs CD4+ T cell responses to influenza A virus (IAV) infection in adult offspring. Moreover, the damped CD4+ T cell response to IAV can be transferred to mice that were not exposed during development. This suggests that these changes are laid down during development, are long lasting, and implicates altered epigenetic regulation as a potential mechanism. Since DNA methylation is an epigenetic system that influences CD4+ T cell proliferation and differentiation, we hypothesize that developmental AhR activation alters immune responses via changes to DNA methylation. We utilized whole genome bisulfite sequencing to map how developmental exposure impacts DNA methylation in CD4+ T cells before and after infection. Developmental exposure results in differential methylation and an enrichment of gene ontology categories related to differentiation and immune response. Differentially methylated regions reflect a combination of hyper- and hypo-methylated regions, and span all genomic features. To determine whether altered DNA methylation is responsible for impaired CD4+ T cell responses, we treated developmentally exposed mice with S-adenosylmethionine (SAM) or zebularine to enhance or decrease DNA methylation, respectively. Following infection, SAM restored the expansion of CD4+ T cells in TCDD exposed offspring. It also reversed the dampened Th1 cell response. Zebularine rescued the diminished frequency of Th1 and Thb, but it did not alleviate suppression of CD4+ T cell expansion. Taken together, these results indicate that altered DNA methylation is a mechanism by which developmental exposures cause durable changes in antiviral immunity, and that hyper- and hypo-methylation regulate distinct aspects of CD4+ T cell responses to infection.

The transcription factor nuclear factor erythroid-derived 2 like-2 (Nrf2) is activated by a number of cellular stressors, such as reactive oxygen species and electrophilic compounds including tert-butylhydroquinone (tBHQ), a widely used food preservative. Upon activation, Nrf2 upregulates many cytoprotective genes, including genes involved in antioxidants defense, detoxification, and phase II metabolism. Nrf2 plays a role in the regulation of immune responses, with an established role in the regulation of inflammation in animal models. Studies have also indicated cell-type specific roles for Nrf2 in immune cells such as dendritic cells, macrophages, and T cells, among others. In murine helper (CD4) T cells, Nrf2 activation skews CD4 T cell differentiation towards a Th2 (allergy-like) phenotype and has differential effects on activation. However, the role of Nrf2 in human CD4 T cells remains unclear. Previous studies demonstrated that the Nrf2 activator tBHQ inhibits events of primary human CD4 T cell activation, but the role of Nrf2 in these effects was not determined. To address this gap, primary human CD4 T cells were transfected with siRNA targeting Nrf2 to generate Nrf2-deficient primary human CD4 T cells. These cells, along with the corresponding scrambled control siRNA -transfected cells, were used to determine the role of Nrf2 in tBHQ-mediated effects on early T cell activation. tBHQ treatment followed by T cell activation with antiCD3 antiCD28 inhibited induction of the cytokines IL-2, IFNγ, GM-CSF, and TNFα, and the cell surface proteins CD25 and CD69 in both scrambled control and Nrf2-targeted siRNA transfected cells, suggesting these effects are Nrf2 independent. RNA-seq analysis identified differentially expressed genes between the scrambled control and Nrf2-deficient cells, suggesting there are genotype differences in early T cell activation. In addition, the RNA-seq analysis showed Nrf2+ independent and Nrf2-dependent effects of tBHQ at the RNA level. This indicates a potential role for Nrf2 in aspects of primary human T cell activation and differentiation. This work is funded by NIH grants ES018885, ES023755 and ES024966.
3245 Lipopolysaccharides Increased the Expressions of Interleukin-8 and Interleukin 1-Beta in the Macrophages Differentiated from ML-1 Monocytes and Upregulation of Cellular Antioxidants Reduced Lipopolysaccharides–Induced Inflammation


Sepsis is a condition known to damage bodily tissues and organs caused by a dysregulated response to infection by the body. It causes millions of deaths annually throughout the world and is the primary cause of death in hospitalized patients. However, the precise mechanisms underlying this illness remain to be defined. Macrophages have been suggested to play an essential role in the initiating the cascade of inflammatory cytokines and the pathophysiology of septic shock. In the current study, we used phorbol-12-myristate-13-acetate (PMA) to differentiate the ML-1 monocytes into macrophages as demonstrated by an increase in the expression of Cluster of Differentiation 206 (CD206, a macrophage differentiation marker), and further investigated if macrophage antioxidants would modulate LPS-induced inflammation. Cellular reduced glutathione (GSH) and NAD(P)H:quinone oxidoreductase 1 (NQO1) are two important cellular defenses against oxidative and inflammatory stress. Triterpenoid 2-cyano-3,12-dioxoolean-1,9-diene-28-imidazolide (CDDO-im) was used to upregulate expression of GSH and NQO1. Our data demonstrated treatment with LPS at 100 ng/mL dramatically increased the levels of inflammatory gene expression such as interleukin-1 beta (IL-1beta) and interleukin-8 (IL-8) in the macrophages differentiated from ML-1 monocytes. Interestingly, induction of GSH and NQO1 by CDDO-im afforded protection against the LPS-induced expression of IL-1beta and IL-8 suggesting that GSH and NQO1 may play an important role in the LPS-mediated inflammatory response. Together our data demonstrated that upregulation of endogenous cellular antioxidants could attenuate LPS-induced inflammation. This study may contribute to advancing our understanding of sepsis, a life-threatening illness.

3246 Identification and Characterization of a Sensitive Immunologic Target of TCDD: CDS+ Innate-Like B Cells

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Xenobiotic-mediated activation of the aryl hydrocarbon receptor (AhR) is immunotoxic in a number of immune cell types, with the B cell being a well-established sensitive target. Recent advances in immunology have provided evidence that the B cell repertoire is a heterogeneous population, with subpopulations exhibiting vastly different cellular and functional phenotypes. Recent work from our laboratory identified the T cell specific kinase lck as being differentially regulated by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which is a potent activator of AhR. While lck is primarily expressed in T cells, a broad class of B lymphocytes termed innate-like B cells (ILBs) which are critical mediators of innate immunity through constitutive secretion of polyvalent natural immunoglobulin M (IgM). We hypothesized that CDS+ ILBs may be sensitive to AhR-mediated immunotoxicity. Indeed, when CDS+ B cells were isolated from the TCDD-treated pool and treated with TCDD, they showed increased expression of CDS, CD5, and interleukin-8 (IL-8) in response compared to CDS+ B cells. Further, characterization of the CDS+ population indicated increased basal expression of AhR and cytochrome p450 family 1 member a1 (Cyp1a1). Indeed, the levels of AhR-mediated suppression of the IgM response from individual donors strongly correlated with the percentage of the B cell pool that were CDS+, suggesting that CDS+ B cells are more sensitive to AhR-mediated immunotoxicity. Together these data highlight the sensitive nature of CDS+ ILBs to AhR activation and provide insight into mechanisms associated with AhR activation in human B cells.

3247 The Synthetic Food Additive TBHQ Impairs Host-Defense to Influenza Infection


In the 2017/18 flu season, the United States had 80,000 deaths attributed to influenza virus infection. Despite enhanced vaccination efforts, influenza infection rates and related deaths have not improved, suggesting a disconnect between clinical efforts and patient outcomes. Therefore, it is important to consider other factors that may contribute to worsened influenza burden and poor vaccine efficacy. We previously showed that the widely-used food additive butylhydroxyanisole (BHA), impairs post-infection cytokine secretion in T cells and T1 cell polarization (important in anti-viral defense) through activation of NFκB, a stress-activated transcription factor. Accordingly, we hypothesized that TBHQ consumption would impair host-defense against influenza virus infection. To test this hypothesis, we fed C57BL/6J mice control or TBHQ-containing diets prior to infection with influenza A/PB/1934 (H1N1). TBHQ consumption reduced cytotoxic T cell infiltration in the lung, CD4/CD8 T cell activation and effector function, influenza-specific T cell numbers, and viral clearance. Additionally, TBHQ substantially increased expression of the inhibitory receptor, CTLA-4, providing a novel mechanism by which TBHQ can suppress T cell activation. Upon primary exposure to influenza, weight loss was unaffected by TBHQ consumption. However, the diminished T cell response by TBHQ led to exaggerated weight loss and prolonged recovery in a secondary infection model, suggesting that TBHQ impaired the memory response to influenza infection. It should be noted that the NOAEL used to establish the allowable daily intake (ADI) of TBHQ is 72 mg/kg/day, and the current study’s dose was between 1-3 mg/kg/day, suggesting a need to re-evaluate the current ADI. Moreover, the doses used in our studies are easily achievable with a western diet. Overall, our studies show that consumption of a physiologically-relevant dose of TBHQ impairs the primary immune response to influenza virus infection leading to worsened outcome during secondary infection, which would be expected to result in poor vaccine efficacy.

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3248 A Multi-Stakeholder Dialogue on Using Proprietary Modeling Platforms to Support Risk Assessment and Regulatory Decisions

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Powerful modeling platforms embedded with large databases allow researchers to more efficiently generate robust predictions to evaluate a specific endpoint (e.g., potential toxicity of a compound) even when little data are available. For example, several proprietary physiologically based pharmacokinetic (PBPK) modeling platforms contain extensive demographic, physiologic, and biochemical databases, and simulate internal dosimetry of a compound to assist in extrapolations necessary to estimating human health risks from exposures to environmental chemicals, or evaluating safety and efficacy of drug compounds. Being able to predict human relevance is of keen interest to diverse groups including regulatory agencies that use PBPK models to determine safety of chemicals, drugs, and consumer products; regulators; and the general public. Proprietary models have been used extensively in certain regulatory arenas, such as drug development, and are new to others, such as pesticide registration. The primary concern that prevents a regulatory agency from incorporating predictions generated from proprietary modeling platforms into their decision-making processes is the lack of open access to the platforms and underlying models and databases. In this case, regulators, academics, or members of the general public may not be able to replicate the model predictions, or to evaluate the predictive capability of the model within a desired chemical space. This roundtable assembles model developers, model users (e.g., chemical registrants and academics), risk assessors within regulatory agencies, and external stakeholders to share their experience and perspectives on both scientific and nonscientific challenges that limit the use of proprietary modeling platforms in regulatory assessment. While this session will focus the discussions on PBPK modeling platforms, the perspectives are applicable to other modeling platforms. The session will start with brief presentations of different perspectives on the utility of proprietary modeling platforms in both development and safety assessment of chemicals, drugs, or consumer products; processes involved in validating/evaluating model assumptions and predictions; and considerations for protecting intellectual property. Following the presentations, there will be a 50-minute moderated discussion among presenters and audience addressing questions such as, “Is evaluation of a model by the platform developer sufficient to provide confidence in the outputs of a model?” and, “Is access to all modeling platforms and databases used to generate model outputs required to verify the output?” This roundtable gathers parties involved in the use of proprietary models to explore strategies that...
Nerve Agent and Pesticide Poisoning: Best Practice Methodologies for Assessing Long-Term Health Effects

L. Roszell, Army Public Health Center, Aberdeen Proving Ground, MD.

Exposure to nerve agents, such as chemical warfare agents and organophosphorous pesticides, is a highly topical subject in toxicology, unfortunately because of their recent use in civilian and military conflict. Nerve agent poisoning after acute high doses is often fatal; however, if life-threatening symptoms can be controlled through medical intervention, many people can survive the acute lethal toxicity. There is an existing body of literature that strongly suggests that nonlethal adverse health effects occur in survivors of acute nerve agent exposure. These "long-term" health effects include neurochemical, neuropathological, and behavioral deficits that occur within days, weeks, or even many years after the exposure. The acute lethal effects of organophosphorous (OP) nerve agents and pesticides have been well described, and data exist for the assessment of hazard and risk. However, understanding and assessing the risk of long-term sequelae is less clear due to the heterogeneity and rigor of human and animal studies. Data on the long-term effects are also important for developing effective medical interventions, since some of the studies describe persistent effects that can significantly reduce quality of life. Often risk assessments are largely retrospective, relying on qualitative data by estimating signs and symptoms at the time of exposure and how long it took for them to develop. The session will present examples of methods for assessing long-term effects in humans and animals following acute exposures to OP nerve agents and pesticides. The session will begin with an overview of the issue, including examples of significant incidents and efforts to retrospectively link exposures to outcomes. The next presenter will describe an NIH/NTP Systematic Review of long-term neurological effects of sarin. This will be followed by a presentation describing a toxidrome-based, subject-matter expert (SME)-informed approach for assessing the risk of long-term health effects following acute exposures to OP nerve agents and pesticides, and the fourth presentation will discuss preclinical models for assessing the neuro-pathological changes induced by acute intoxication with OPs, and the short- and long-term functional deficits associated with the acute exposures. The session will conclude with a panel discussion.

Overview: Why Do We Need to Understand Long-Term Adverse Health Outcomes following Acute Exposures to Nerve Agents?

L. E. Roszell, Army Public Health Center, Aberdeen Proving Ground, MD.

The issue of long-term health effects caused by acute chemical exposures is relevant to both military and civilian populations. From a military perspective, understanding such risks would assist planners in better assessing the overall health risk of a mission and assist medical personnel in the long-term care of soldiers. Similarly, from a civilian perspective, understanding would improve health risk assessment capabilities associated with accidental or intentional exposures as well as assist medical personnel in the long-term care of civilians. Acute exposures may be the result of an intentional or unintentional chemical release. For example, twelve people were killed and many more injured when sarin was released on the Tokyo Metro in 1995. More recently, both civilians and soldiers were exposed to the organophosphorus (OP) nerve agent sarin when it was used as a weapon in the Syrian conflict. There are also numerous reports of agricultural workers acutely poisoned with OP pesticides. A major question is whether there are long-term adverse health effects subsequent to surviving sublethal acute exposures to OP chemicals. Because most studies to date have been retrospective it is difficult to definitively link illness to exposure. Current efforts to address this issue include systematic literature reviews and laboratory studies. If adverse effects are believed to be a result of OP exposure, a critical follow-on question relates to the severity of the long-term effect; more severe effects might require greater resources to manage and treat. Filling these data gaps will provide information that can be used by military planners to assess overall risk to US forces, and by civilian planners to inform long term risk and medical care.

A Systematic Approach for Assessing Long-Term Effects of Sarin

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Several literature reviews of the long-term neurological effects following exposure to sarin have been published. However, a systematic review of the evidence has not been performed in which selection criteria were clearly stated and consistently applied; where a broad hierarchy of evidence is considered including uncontrolled studies and case-reports or case series; and in which individual studies were assessed for internal validity or risk of bias. A review of data on long-term effects of acute exposure has also never been done where the search was narrowed to eliminate confounding elements such as the possibility of multiple chronic exposures to the agent and exposure to other agents, and where the focus is on a specific set of outcome measures (neurological). The National Institutes of Health (NIH) is conducting a systematic review that focuses on the neurological sequelae following acute exposure to sarin nerve agent. This review is conducted in partnership with several NIH Institutes, including the National Institute of Neurological Disorders and Stroke (NINDS), which leads the NIH CounterACT program, and the National Institute of Environmental Health Sciences (NIEHS), National Toxicology Program, Office of Health Assessment and Translation (OHAT), which is providing expertise in the conduct of systematic reviews for environmental hazards. The specific literature search is led by informatics specialists from the NIH Library who also have substantial experience in the conduct of systematic reviews. Early results confirm the heterogeneity of the data among different studies, and difficulty in finding common data elements among human, animal, and mechanistic data streams. Despite these and other caveats, the results suggest there are long-term neurological effects of acute sarin exposure in humans and animal studies, but there are uncertainties in the existing data, and several data gaps yet to be filled. This systematic review will help understand better the potential long-term effects of organophosphorous nerve agent and pesticides.

A Novel SME-Informed Approach to Assess the Likelihood of Long-Term Injury following Acute Exposures

K. Wegman, Battelle Memorial Institute, Columbus, OH. Sponsor: L. Roszell

The acute effects of many chemicals are well described with supporting data through traditional toxicology studies. In contrast, long-term health effects resulting from a single acute exposure are sparsely supported by anecdotal data. Assessing the risk of long-term sequelae depends entirely on qualitative data or limited data sets with severe limitations. To overcome these un-
certainities and limitations, a novel toxidrome-based, subject matter expert (SME)-informed approach was developed to derive long-term health effect estimates for the cholinergic toxidrome based on the currently available data. Using the Delphi Method, ten SME estimates were obtained for the likelihood of long-term mild, moderate or severe health effects following an acute mild, moderate, severe, or life-threatening health effect from an acute exposure. Responses from all SMEs were combined into a representative consensus and reviewed by all SMEs for approval. The consensus SME input was then linked with chemical-specific, acute dose-response data for chemicals from the cholinergic toxidrome to yield chemical-specific, long-term health effect curves. This toxidrome-based approach allows for applicability across a range of chemicals by applying the toxidrome level results to individual chemicals with similar acute clinical signs and symptoms. The results of this approach provide risk assessors and planners with a method to estimate long-term health effects following acute chemical exposures until more robust data are available.

### Preclinical Models to Assess Long-Term Neurological Sequelae of Acute Intoxication with Organophosphate Nerve Agents

P. J. Lein, University of California Davis, Davis, CA.

Preclinical models are critical for corroborating clinical and epidemiological data and for identifying mechanisms and biomarkers of disease. Given the challenges in studying in vivo health effects of acute, sublethal exposures to chemical warfare agents, the development of relevant preclinical models has been an active area of research. This talk will: (1) provide an overview of the various models that have been developed to assess the long-term neurological sequelae of acute intoxication with OP nerve agents and OP pesticides, including in vitro and in vivo models; (2) describe the short- and long-term functional deficits that have been described in these models following acute OP intoxication, which range from molecular effects (calcium dysregulation) to neuropathology (neurodegeneration and neuroinflammation) to behavioral deficits, including recurrent seizures; and (3) discuss the relevance, and limitations, of these models to the human condition, including how data from animal models may be useful in identifying critical gaps in the human literature.

### New Mechanistic Insights into Causes and Outcomes of Epigenetic Dysregulation by Carcinogenic Metals

C. Jin, New York University School of Medicine, New York, NY.

Metal contamination impacts hundreds of millions of people in the world. Metal exposure can cause human diseases including cancer. Carcinogenic metals are in general considered to be weak mutagens, suggesting that mechanisms other than genetic changes play major roles in metal-induced carcinogenesis. Epigenetic mechanisms have recently emerged as important players in response to metal exposure. Epigenetic regulations include DNA methylation, histone modifications, microRNA expression, incorporation of histone variants, and nucleosome positioning and chromatin accessibility. Most studies of metal-induced epigenetic dysregulation have focused on changes in epigenetic profiles in terms of DNA methylation, global histone modifications, and microRNA expression. However, mechanisms that control these changes and consequences of these changes are not well examined. Moreover, little is known about genome-wide changes in chromatin accessibility and assembly of variant histones following metal exposures. While high-throughput sequencing technologies such as RNA-seq, CHIP-seq, and Methyl-seq have recently been applied for studies of metal-induced epigenetic regulation, newer technologies such as ATAC (Assay for Transposase-Accessible Chromatin) have not been as widely used in studies involving metal toxicity. This symposium aims to highlight recent advances in environmental epigenetics, focusing on new molecular insights into epigenetic dysregulation by metal exposure and on the use of cutting-edge new technologies in studies of environmental epigenetics. The first speaker identifies a set of differentially methylated genes in exfoliated urothelial cells (EUCs) in a cohort study. Promoter analysis shows that the arsenic-associated genes are enriched for the binding sites of canonical histone transcription factors known to play roles in carcinogenesis. The second speaker demonstrates hsa-miR-186 induction by arsenic exposure and how overexpression of hsa-miR-186 induces chromosomal instability in keratinocytes, providing a mechanism for induction of aneuploidy by arsenic exposure. In the third presentation, the speaker presents the changes in the levels of histone variants during arsenic-induced epithelial to mesenchymal transition (EMT) as well as a possible mechanism that causes differential methylation at specific genomic loci in arsenic-transformed cells. The fourth speaker uses cutting-edge new technologies such as DANPOS (Dynamic Analysis of Nucleosome Positioning and Occupancy by Sequencing) and ATAC (Assay for Transposase-Accessible Chromatin) to show how nucleosome positioning and chromatin accessibility are changed by chromium exposure. The fifth speaker uses an animal model to demonstrate polyadenylation of canonical histone mRNAs following nickel and arsenic exposures. In vitro studies further reveal that increase in polyadenylated canonical histone mRNAs disrupts nucleosome assembly of histone variants at active promoters. In summary, this symposium will provide attendees mechanistic and new aspects of epigenetic dysregulation by metal exposure and their implications in metal-induced carcinogenesis, as well as better understanding of new approaches for studying chromatin landscape following environmental exposures. Potential use of these epigenetic changes in cancer risk assessment will be discussed.

### Links between Arsenic-Associated DNA Methylation and Bladder Cancer

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Human population studies support that chronic exposure to inorganic arsenic (iAs) is associated with cancer of the bladder. This health effect, like many related to iAs, may be tied to epigenetic modification. In a cohort in Chihuahua, Mexico, we examined DNA methylation patterns associated with iAs and its metabolites in exfoliated urothelial cells (EUCs) that originate primarily from the urinary bladder, one of the targets of arsenic-induced carcinogenesis. A genome-wide, gene-specific strategy was used to assess promoter DNA methylation levels in EUCs from study subjects. The relationship between promoter methylation profiles and the intracellular concentrations of total arsenic and arsenic species was examined. A set of 49 differentially methylated genes (DMGs) was identified with increased promoter methylation associated with EUC tAs, iAs, and/or monomethylated As (MMAs) enriched for their roles in metabolic disease and cancer. Further analysis showed that a subset of the 49 arsenic-associated genes are also differentially methylated in bladder cancer tissue using The Cancer Genome Atlas (TCGA) repository. Both the arsenic- and cancer-associated genes are enriched for the binding sites of common transcription factors known to play roles in carcinogenesis, demonstrating a novel potential mechanistic link between iAs exposure, the DNA methylome and bladder cancer.

### hsa-miR-186 Overexpression Induces Aneuploidy in Human Keratinocytes

J. States, University of Louisville, Louisville, KY.

Arsenic exposure is known to induce aneuploidy and aneuploidy can play a major role in carcinogenesis. miRNAs can act as oncogenes or tumor suppressors and thus dysregulated expression can play a role in carcinogenesis. Linkage between dysregulated miRNA expression and aneuploidy is unknown. We have observed that hsa-miR-186 is overexpressed in squamous cell carcinoma samples from patients chronically exposed to arsenic in drinking water. The effect of hsa-miR-186 overexpression in keratinocytes on chromosomal stability was investigated in human keratinocytes. Ectopic expression induced an increase in fraction of cells with supernumerary chromosomes and also increased chromosomal structural abnormalities. Overexpression of hsa-miR-186 combined with incubation with 100 nM sodium arsenite increased the frequency of double minutes which are associated with gene amplification. hsa-miR-186 is an intronic miRNA embedded within the ZRANB2 gene. Further investigation on mechanism of hsa-miR-186 induction suggests that arsenite disruption of zinc fingers in ZRANB2 may result in increased transcription of the parent gene in which hsa-miR-186 is embedded. These results are consistent with and may provide a mechanism for induction of aneuploidy by chronic arsenic exposure.

### Chromatin Structural Changes and Function in Inorganic Arsenic-Mediated Cellular Transformation

Y. Fondue-Mittendorf, University of Kentucky, Lexington, KY. Sponsor: C. Jin

Inorganic arsenic (iAs) is a ubiquitous environmental toxicant implicated in carcinogenesis. 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 or epithelial to mesenchymal transition (EMT). Two high-resolution methods were used to profile these epigenetic changes: top-down mass spectrometry to quantify the changes in the levels of H2B variants; and Methyl-seq to identify DNA methylation changes. iAs exposure induces car-
Human exposure to well-established carcinogenic metals nickel and arsenic is associated with increased incidences of various cancers including lung cancer. Although these metals have long been known to induce toxicity and carcinogenicity via epigenetic mechanisms, alteration in histone gene expression is scarcely studied. Canonical histone mRNAs are unique in that they are the only mRNAs in multicellular organisms that do not contain poly(A) tail in their 3′ ends and thus are unstable. Instead, they contain a conserved 26-nucleotide sequence in their 3′ ends, which is the binding site for the Stem-loop binding protein (SLBP). SLBP is essential for processing of the canonical histone pre-mRNA and the loss of SLBP has been shown to induce aberrant polyadenylation of canonical histone mRNAs. Previously we demonstrated that arsenic exposure induces polyadenylation of H3.1 mRNA (a canonical histone H3) in cells due to the loss of SLBP, which appeared to be attributable to arsenic-induced degradation of SLBP and an increase in poly(A)-containing H3.1 mRNA. Intriguingly, increase in polyadenylated H3.1 mRNA compromises assembly of H3.3 (a variant histone H3) nucleosomes at promoters of 2,000 most active genes. In addition, we found that transcription of histone H3.1 with a poly(A) tail causes a G2/M block in the cell cycle, chromosome instability, and DNA damage. To further distinguish between G2 and M arrest, we determined the cellular level of histone H3S10 phosphorylation, a mitotic phase specific modification, by flow cytometry assay. The phosphorylation of H3S10 was increased from 4.6% to 11% upon transcription of H3.1 poly(A) as compared with the control. We further found that transcription of H3.1 with a poly(A)-coding sequence induces a high incidence of cell transformation and these transformed cells form tumors in athymic nude mice. Furthermore, arsenic exposure of mice by inhalation results in the loss of SLBP and gain of polyadenylated H3.1 mRNA in lung tissues. These results suggest that the polyadenylation of canonical histone mRNAs is disruptive to chromatin structure and genomic integrity and may contribute to both arsenic- and nickel-induced carcinogenesis.
3263 Testicular Toxicants as Modifiers of Sperm Epigenetic States: Ethylene Glycol Monomethyl Ether as a Case Study

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Many male reproductive toxicants adversely affect fertility through mechanistic targets in one or more distinct cell types in the testis. Regardless of the primary cell targeted by a testicular toxicant, the common thread among these compounds is impairment of spermatogenesis and/or sperm quality. Ethylene glycol monomethyl ether (EGME) targets a specific germ cell subset, the primary spermatocytes, and leads to germ cell apoptosis at high levels of exposure. We hypothesize that the sperm that develop from EGME-exposed germ cells, and their epigenetic contents delivered to an oocyte upon fertilization, may be compromised. We used 0, 50, 60 and 75 mg/kg EGME for 5 days as a case study, measuring small RNAs in sperm by next-generation sequencing 5 weeks after exposure. Our data illustrate dose-dependent changes in the small RNA populations after EGME exposure with significant increases in mRNA fragments and micro-RNA populations. Many of the individual mRNA fragments are decreased, specifically in the RNA population cellular survival and spermatogenesis pathways. However, there was a dose-dependent increase in number of unique mRNA fragments after EGME exposure. Many Piwi-associated RNAs (piRNAs) were differentially expressed with treatment, and there was a significant increase with EGME exposure in the number of these piRNAs that aligned to transposable elements. The small RNA endpoints have lower BMD values compared to histopathology or sperm motility endpoints. This study provides a mechanistic basis to link preconception exposure to EGME on offspring development and health through sperm quality.

3264 Male Preconception Phthalates on Sperm Epigenetics and Early-Life Development

J. Pilsner. University of Massachusetts Amherst, Amherst, MA. Sponsor: A. Timme-Laragy

Compelling data demonstrate that preconception environmental exposures can be embodied within the developing male germ cell through epigenetic marks. In turn, these epigenetic marks may impart information at fertilization to affect the trajectory of offspring health and development. Phthalates, a class of endocrine disrupting compounds (EDCs) used in plastics and personal care products, are ubiquitous environmental contaminants resulting in widespread human exposure. Phthalate exposures have been reported to cause a host of behavioral and reproductive health issues. In particular, phthalate exposure in males is associated with poor sperm quality, and more recently, with longer time to pregnancy, which suggests a sperm-derived effect. The proposed oral presentation at SOT 2019 will focus on our results from two ongoing studies; 1) our human cohort, Sperm Environmental Epigenetics and Development Study (SEEDS), and 2) our mouse model system, which utilizes divergent genetic backgrounds of parental mice that allows for allele-specific examination of DNA methylation in F1 offspring. Utilizing genome-wide DNA methylation approaches, we will present our findings on the effect of preconception phthalate exposure on sperm methylation and early-life development. Our findings in SEEDS show that male preconception anti-androgenic phthalates are associated with sperm DMRs and subsequent blastocyst quality. Additionally, our mouse data support our human findings, such that di(2-ethylhexyl) phthalate (DEHP) exposure results in altered DNA methylation in sperm and gene expression in F1 hybrid embryos. These results suggest that paternal adult environmental conditions may influence epigenetic reprogramming during spermatogenesis, and in turn, influence early-life development.

3265 Maternal Preconception Exposure to PFOS Affects Nutrient Content of Oocytes and Later-Life Pancreas Development

K. Sant. San Diego State University, San Diego, CA.

Before the placenta becomes fully functional late in the first trimester, the human embryo’s primary source of nutrients is the yolk—a cache of maternally-deposited lipids and proteins. The yolk quantity and composition deposited in oocytes can be disrupted by maternal exposures to environmental toxicants, with potentially profound impacts on the developing embryo and later-life health. In this study we used zebrafish (D. rerio), the nematode worm (C. elegans) and the fruitfly (D. melanogaster) to identify evolutionarily conserved changes in egg nutrient composition and later-life health following maternal exposure to the legacy toxicant perfluorooctanesulfonic acid (PFOS). We have shown that maternal perfluorooctane sulfonate exposure in the zebrafish altered nutrients loaded in to the oocytes resulting in lower amounts of protein and cholesterol. These exposures also impaired pancreatic organogenesis during the embryonic stage of the fish. In the fly we found that exposure of female adults to PFOS resulted in reduced egg numbers per female, a response reflective of inadequate nutrition in the egg chamber, and also a reduced expression of megalin, a gene that encodes for an important transporter of nutrients into the oocyte. In the worm, we also found that preconception exposure results in later-life elevation of triglycerides in the gonad, suggesting potential metabolic dysfunction. Overall, these data indicate that maternal preconception exposures can reduce oocyte quality and impair metabolic function at later-life stages.

3266 Does Exposure to Mitochondrial Toxicants during Germ Cell Development Result in Lifelong Alterations in Mitochondrial Function Mediated by Epigenetic Changes?

J. Meyer. Duke University, Durham, NC.

Growing evidence suggests that the toxic effects of certain chemicals on mitochondrial function can be highly persistent. This is critical because mitochondrial function influences organismal phenotypes related to chronic diseases such as metabolic diseases, cancers, neurodegenerative diseases, and reproductive disorders. The likelihood of persistent effects may be especially great for exposures of germ cells and gametes, because mitochondria undergo biogenesis and major functional changes during germine proliferation and gamete production. Furthermore, epigenetic patterns that can have long-term effects on cellular function are reprogrammed in the same timeframe. Using the model organism Caenorhabditis elegans, we are testing the hypothesis that pollutant exposures targeting mitochondria in germ cells result in persistent changes to histone modification that escape embryonic reprogramming and alter regulation of pathways governing mitochondrial metabolism in offspring. To date, we have found that developmental exposures to rotenone (a complex I inhibitor) and arsenic (which inhibits the function of multiple mitochondrial as well as non-mitochondrial proteins) can result in lifelong alterations in mitochondrial function (in particular, oxygen consumption) and organismal fitness. Metabolomic and genetic analysis of alterations resulting from arsenic-induced exposure suggest that this is a result of persistent alterations in insulin and mitochondrial oxidative stress pathways. In the case of rotenone, preliminary data (two replicates) demonstrated that F1 offspring of exposed (preconception) parents had 50—75% decreases in ATP-linked oxygen consumption, as well as decreases in basal and maximal, but not non-mitochondrial, oxygen consumption. This was true at all exposure levels, including levels that had no effect on growth.

3267 Potential Alternatives to Systematic Reviews: Evidence Maps and Scoping Reviews

B. Beverly. NIEHS, Research Triangle Park, NC.

Systematic reviews, with their comprehensive, transparent, and repeatable protocols, are rapidly becoming the gold standard. However, there is literature to assess environmental health questions. However, there are instances when a full systematic review may not be feasible or necessary to address particular questions or to make decisions (e.g., for funding, future research, or regulation). Interestingly, there are a variety of methods that can be used to answer questions in environmental health data that do not require (or are premature for) an exhaustive systematic review. Scoping reviews and evidence maps are systematic, transparent assessments that may be better suited for some research questions. These intermediate alternative products can be performed more quickly and are often used to inform systematic reviews (usually in the initial steps of problem formulation and development), and can also serve as publishable stand-alone products. Two major challenges of conducting systematic review for decision-making are the time and resources needed to complete the review. On average, systematic reviews can take more than 1,000 hours to complete and can cost over $100,000. Further, adult stage, suggestive of metabolic dysfunction. Overall, these data indicate that maternal preconception exposures can reduce oocyte quality and impair metabolic function at later-life stages.
3268 Brief Overview and Introduction to Systematic Review and Related Products

B. Beverly, NIEHS/NTP, Research Triangle Park, NC.

Systematic review is rapidly becoming the gold standard for addressing environmental health questions. The systematic review process involves the objective and transparent method of collecting and synthesizing data for reaching hazard conclusions on specific research questions. This overview will provide a brief review of systematic review methodology and the relatively recent uptake of systematic review practices from different environmental health groups. This overview will highlight the strengths and challenges of conducting systematic review in addition to briefly introducing the intermediate products (systematic maps and scoping studies) that can be used for decision-making for environmental health questions that will be discussed in subsequent presentations.

3269 Rigor and Resources for Systematic Reviews in Toxicology: Case Study Applications in Food Safety, Consumer Product Safety, and Environmental Health Risk Assessment

D. Wikoff, ToxStrategies, Inc., Asheville, NC.

Systematic review is a type of rigorous, evidence-based analysis for answering a specific research question. This methodology is rapidly being implemented globally in the field of toxicology, though is often met with resistance due to the resources required to conduct a high quality review. The objective of this presentation is to provide a demonstration of the rigor and resources required to conduct full systematic reviews in toxicology with the aim of helping practitioners to determine when such a review is needed. A systematic review of caffeine safety in healthy adults, pregnant women, adolescents, and children will be used as a case study to demonstrate key elements of conducting a systematic review according to standards from the Institute of Medicine. Broad in scope, this systematic review addresses five PECO questions and integrates >380 papers to develop conclusions. This case study will highlight the role of problem formulation in determining if and when a systematic review is needed, as well as the protocol development and topic-specific refinements needed for the application of systematic review methodologies originally developed for clinical medicine. The case study on caffeine will also address the utility of multidisciplinary teams, implementation of a priori methods, and application of systematic review to a very large evidence base. It will also highlight the need for refined guidance and frameworks unique to the conduct of systematic review (e.g., multi-endpoint reviews) in the fields of toxicology and risk assessment. Using other case studies, additional elements of systematic review which are commonly underappreciated will be addressed, including appraisal and integration of study validity via risk of bias, evaluation and integration of multi-stream evidence bases ([including mechanistic data], and application of such concepts in qualitative vs. quantitative assessments). Collectively, the case studies will characterize the rigor and resources required for the conduct of high quality systematic reviews—practical information needed for toxicologists and risk assessors to understand the benefits and challenges of conducting meaningful systematic reviews in the fields of toxicology and risk assessment.

3270 Systematic Mapping as a Tool for Regulatory Risk Assessment in Environmental Health: Tetrabromobisphenol A (TBBPA) as a Proof of Concept

T. Harrison. Lancaster University, Lancaster, United Kingdom. Sponsor: B. Beverly

Tetrabromobisphenol A (TBBPA) is the highest production volume brominated flame retardant on the market, finding extensive application in printed circuit boards and polymers. Regulatory interest in TBBPA can be found in its listing as a Toxic Substances Control Act (TSCA) work plan chemical in the United States, and in its selection as a Community Rolling Action Plan (CoRAP) Chemical in the European Union, making it an ideal candidate for exploration of the potential and practicalities of systematic mapping for regulatory decision-making. Key lessons learned from the construction of a proof-of-concept systematic map on the human health risks posed by TBBPA and the long-term implications for the conduct of chemical risk assessment are presented. These include how the production of a context-neutral evidence map necessitates development and implementation of new database techniques not previously used in systematic reviews or systematic maps, but long familiar in computer science. In particular, the value of graph databases—the technology underpinning ultra-connected platforms such as Google, Twitter and Facebook—for capturing and enabling the querying of complex relationships in large toxicological datasets will be discussed. Storing data in network-like graph structures has significant implications for enabling AI augmented probabilistic chemical risk assessment. Very large datasets will allow AI to describe the statistical “shape” in evidence space of toxicity concepts such as carcinogenicity, and then compare this to the “shape” of individual substances in that space. Resulting in a probabilistic description of the extent to which a substance can be said to be known as carcinogenic.

3271 Illustrating Fit for Purpose in Systematic Evidence Maps: Case Study Mapping of the Evidence of Transgenerational Health Effects

V. Walker. NIEHS/NTP, Research Triangle Park, NC. Sponsor: B. Beverly

Evidence maps are a new method in environmental health science used to categorize literature and rapidly map key concepts, types of evidence, and gaps in research related to a defined topic. Mapping displays the extent of literature with varying degrees of detail depending on topic and goals. A detailed scoping review of the frequently debated issue of transgenerational inheritance exemplifies the fit for purpose approach for evidence mapping and illustrates the range of complexities that can be mapped. While an increasing number of reports suggest early life exposures result in adverse effects in offspring who were never directly exposed (or “transgenerational inheritance”), attempts to synthesize findings across studies are complicated by the lack of consistent terminology and diverse evidence base. A simple evidence map for transgenerational inheritance by health effects, exposures, and evidence streams demonstrates the utility of a low detail, rapid mapping for identifying data gaps and more robust pockets of evidence. With in-depth mapping, consideration of study quality and reporting factors is layered onto the existing map as an example of more complex categorization. We illustrate systematic mapping as a flexible tool to assist problem formulation, more efficiently focus resources and facilitate evidence synthesis in regulatory decision-making.

3272 Using Scoping Reviews to Guide Systematic Reviews and Future Research

C. Kwiatkowski. The Endocrine Disruption Exchange, Eckert, CO. Sponsor: J. Rochester

Many of the challenges of conducting systematic reviews in environmental health and toxicology can be addressed by the use of systematic scoping techniques. Scoping provides data to plan the protocol and frame the research question. It identifies specific endpoints and exposures that have sufficient evidence for systematic review. Scoping is also useful for identifying research gaps that can be addressed in the design of future studies. TEDX recently published several scoping reviews that demonstrate the role of the scoping process. They included many features of systematic review, such as a planned protocol with a PECO statement, rapid scoping, a context-neutral evidence map for transgenerational inheritance by health effects, exposures, and evidence streams, consideration of study quality and reporting factors. TEDX recently published several scoping reviews that demonstrate the role of the scoping process. They included many features of systematic review, such as a planned protocol with a PECO statement, rapid scoping, a context-neutral evidence map for transgenerational inheritance by health effects, exposures, and evidence streams, consideration of study quality and reporting factors. Data extraction included the number and age of subjects, the models used (e.g. human, rodent, fish), exposure routes and duration, doses/concentrations measured, and outcomes assessed. Unlike systematic reviews, they did not assess individual studies for quality
Inhalation is a major route of human exposure to airborne substances. This may cause portal-of-entry effects in the respiratory tract and can also lead to systemic uptake and subsequent effects. Several adverse outcomes in the airways are known, including acute lethal effects and chronic diseases. Adverse outcome pathways (AOPs) can be used to describe the mechanism through which a substance causes toxicity and inform the selection of in silico and in vitro methods to include in integrated approaches to testing and assessment (IATAs). In this session, speakers from government, industry, academia, and NGO will discuss how mechanistic risk assessments reflecting inhalation exposure, including lung inflammation and irritation, and the in silico and in vitro methods that can be used to assess key events. Case study examples showing how AOPs have been used to design testing approaches that inform risk assessment decisions will be highlighted. The presentations will discuss the implementation of IATAs that combine the use of existing data with dosimetry considerations, physicochemical property information, in vitro, and computational approaches to fulfill current data needs. Specifically, the first presentation will set the stage for the remaining talks by highlighting the importance of and providing an example of material characterization and dosimetry considerations that must be addressed. The second talk will describe a computational and in vitro approach based on an AOP for squamous metaplasia that has been submitted to the US EPA for the registration of a fungicide. The third talk will discuss an integrated approach for testing reactive gases using an AOP for ILC-2-mediated respiratory remodeling to inform alternative interpretations of study results. The fourth presentation will detail the use of precision-cut lung slices to query key events in an AOP for chronic obstructive pulmonary disease. The final talk will present a regulatory perspective on processes in place to accept alternative approaches for inhalation toxicity testing, the use of mechanistic and exposure information to facilitate regulatory acceptance, and remaining hurdles.

**W 3274 Methods for the Dispersion Preparation and Characterization of Nanomaterials in Physiologically-Relevant Media: Linking Particle Kinetics, Dosimetry, and Bioactivity**

P. Demokritou, Harvard T. H. Chan School of Public Health, Boston, MA.
Sponsor: A. Clippinger

An understanding of cellular dosimetry is essential in the design of studies to assess the toxicity of inhaled substances. As a case study, this presentation will focus on the effects of particle dispersion and characterization of engineered nanomaterials (ENMs) on dosimetry and bioactivity of various classes of ENMs including metal and metal oxides and anisotropic materials.

The engineering of nanomaterials and control of their morphological properties are an essential step in the development of ENMs. There has been a rise in the rise due to their versatile nature and applicability in many industrial fields. Their intrinsic properties depend on the raw material and their manufacturing approach. A thorough physicochemical and morphological characterization of ENMs at the pristine level as well as their dissolution kinetics and transformation in biological and environmental media will be displayed. The regulatory and toxicological implications of these changes are critical for the meaningful assessment of their biological properties. Unfortunately, the description of interactions between carbon-based, anisotropic ENMs and biological and environmental media presents a technical challenge. As a result, there is a lack of standardized methodologies across the dispersion, preparation, and characterization of these materials which can be implemented in cellular studies of their toxicological profile. The objective of this presentation is to present standardized methods for the dispersion preparation and characterization of ENMs, including anisotropic ones, in physiologically relevant media. Specifically, we present methods for the reproducible dispersion and characterization of these materials in water, phosphate buffered saline, and cell culture media. The dispersibility of such ENMs was assessed using light scattering and absorption as well as electron microscopy techniques. The objective was to identify any medium-specific transformations of their interfacial properties and how they might impact their biological properties. Finally, the delivered mass and surface area of anisotropic ENMs to an in vitro cellular system as a function of time was also assessed providing important information in terms of particle kinetics and dosimetry. Such well-defined and standardized methodologies could ensure the safe and sustainable use of emerging ENMs, enable the cross-comparison of toxicological data across laboratories, and represent a key first step in the development of human-relevant integrated testing approaches.
extend the AOP into a network that includes contribution from activation of
TRP channels will also be briefly described. The views expressed in this abstract
are those of the author and do not necessarily represent the views or policies of
the US Environmental Protection Agency.

3277 Ex Vivo Precision-Cut Lung Slices: Highlighting Key Chronic Obstructive
Pulmonary Disease (COPD) Events for Adverse Outcome Pathways
H. Behrsing. Institute for In Vitro Sciences, Inc., Gaithersburg, MD.

Many available in vitro models for human airway exist and can provide var-
ious levels of relevant biological complexity to allow toxicological pathway
assessment. Arguably the most physiologically-relevant non-animal model of
the lower lung (with reasonable throughput), precision-cut lung slices (PCLS)
offer native lung architecture, including small airways and respiratory
parenchyma. These more physiologically-relevant, 3-dimensional tissues that
contain immune-competent cell types such as macrophages and dendritic
cells are often considered essential to the establishment of relevant expos-
sure-induced changes. Tissue conditions such as chronic inflammation are be-
lieved to drive key events involved in diseases leading to chronic obstructive
pulmonary disease (COPD). This presentation will highlight various endpoints
PCLS have exhibited that are also known to be involved in COPD disease pro-
gression. Markers of irritation, inflammation, fibrosis, etc. will be reviewed in
the context of their representation of key events involved in adverse outcome
pathways of lung disease. With an ever-growing scope of inhalable materials
requiring safety testing, robust and relevant non-animal models are essential
to making reliable assessments about risk to human health.

3278 Opportunities to Use Alternative Approaches for Inhalation Risk Assessment of Pesticides

The US Environmental Protection Agency’s (US EPA) Office of Pesticide
Programs (OPP) regulates the use of all pesticide chemicals. To evaluate poten-
cial risks to humans, the OPP evaluates exposures from multiple routes, includ-
ing inhalation, as part of the human health risk assessment. Typically, in
vitro studies are required and/or used to evaluate inhalation exposures: how-
ever, the regulatory statutes provide the US EPA with the flexibility to
modify the actual data and studies required on an individual basis. Therefore,
the Agency may use data from alternative methods and strategies to satisfy
data requirements. The organizing framework for the US EPA’s strategy to
reduce vertebrate animal testing relies heavily on what have been termed
new approach methodologies (NAMs). NAMs have been adopted as a broadly
descriptive reference to any non-animal technology, methodology, approach,
or combination thereof that can be used to provide information on chemi-
cal hazard and risk assessment. There are currently a variety of approaches for
integrating NAMs for regulatory decision making, including defined ap-
proaches, adverse outcome pathways (AOP), and integrated approaches to
testing and assessment (IATA). Furthermore, OPP has developed guidance on
waivers for in vivo subchronic inhalation studies. The waiver guidance uses a
weight of evidence approach that incorporates exposure patterns, physico-
chemical properties, toxicological characterizations, mode of action information
and read across. This presentation will highlight the adoption of waivers and in
vitro mechanistic approaches to inhalation risk assessments and provide
case studies on how understanding the pathways and magnitude of exposure
informs the need for such studies while also discussing the successes and
challenges with replacing in vivo inhalation studies.

3279 Assessment of Cardiovascular Effects following E-vapor and Conventional
Cigarette Smoke Exposure in the ApoE-/- Mouse Model
E. Wong1, J. Szostak2, F. Paneni3, T. Lee1, C. Teng1, K. Lee4, J. Zhang5, P. Leroyp, B. Phillips1, W. McKinney2, M. Peitsch3, P. Vanscheewijk2, and J. Hoeng1, 1PMI R&D, Singapore, Singapore; 2PMI R&D, Neuchatel, Switzerland; 3Center for Molecular Cardiology, Zurich, Switzerland; and 4Altria Client Services LLC, Richmond, VA.

Chronic exposure to cigarette smoke (CS) is a risk factor for the develop-
ment and progression of cardiovascular disease. Considerable attention has
been given to the potential reduced harm of e-vapor products. ApoE-/- mice were
used to evaluate atherosclerosis development, cardiac function, and
aortic stiffness upon exposure to fresh air (Sham), CS from a 3R4F reference
cigarette, or e-vapor aerosols generated using capillary aerosol generators from
various e-vapors ("CARRIER" containing humectants [propylene glycol, glycercin], "BASE" containing humectants and nicotine, and "TEST" contain-
ing humectants, nicotine, and flavors). ApoE-/- mice were exposed for three
hours per day for six months via whole-body inhalation. Measurement of li-
ipoprotein cholesterol concentration, quantification of atherosclerotic plaque
formed, and ultrasound scans of the hearts and aortas were performed. Total serum and very low-density lipoprotein cholesterol were increased in the
3R4F group at Months 3 and 6 but were reduced in the BASE and TEST
groups compared with the Sham or CARRIER groups at Month 6. In contrast
to CS, exposure to any of e-vapor groups did not increase aortic plaque for-
mation when compared with the Sham group. CS exposure was associated
with an impairment of both systolic and diastolic cardiac function, as assessed
by ejection fraction, fractional shortening, isovolumic relaxation time, and
E/A ratio. A subtle impairment of systolic-diastolic performance, as assessed by
myocardial performance index, was observed in BASE and TEST groups when
compared with the Sham or CARRIER groups. All groups containing nicotine
(ICS, BASE, and TEST) displayed increased stiffness of abdominal aorta and
carotid artery, with the effect being most prominent in the CS group. In con-
clusion, chronic CS exposure aggravated atherosclerosis, which was not ob-
erved in the e-vapor aerosol-exposed groups. Increased aortic stiffness was
more pronounced in the CS-exposed group as compared with nicotine-con-
taining e-vapor aerosol-exposed groups.

3280 The Role of the TRPA1 Channel and Beta-
Adrenergic Receptors in the Arrhythmogenic
and Autonomic Effects of E-cigarette Aerosols
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Louisville School of Medicine, Louisville, KY.

Electronic cigarette (e-cig) use has increased in prevalence, especially among
youth. E-cigs heat liquid media - dominated by propylene glycol (PG) and
vegetable glycerin (VG) - to generate potentially toxic constituents. Exposures
to e-cig aerosols and their individual constituents have been linked to adverse
cardiopulmonary effects, but the extent of harm and underlying mechanisms
are unknown. The transient receptor potential A1 (TRPA1) channel in airways
is triggered by irritants to activate sensory neurons and may destabilize auto-
nomic regulation to increase cardiac risk. We sought to elucidate the role of
TRPA1 and β-adrenergic receptors (βARs) in the cardiac effects of e-cig aero-
sols. Electrocardiograms (ECGs) were collected by radiotelemetry in TRPA1
knockout (KO, n=4) and wild type (WT, n=4) mice and analyzed for heart
rate variability (HRV) and repolarization (QT, and J amplitude). After sham
exposure (Air, 90-min), mice were acutely exposed on a separate day to e-cig
aerosols (three 9-min exposures) from equal-ratio PG and VG (PG:VG) and
were subsequently pretreated with a β-blocker (propranolol) and exposed to
PG:VG again. During exposure, PG:VG markedly decreased heart rate (HR) and
increased time-domain HRV parameters in WT while decreasing J amplitude
(all P<0.05 vs. Air) and inducing frequent bradyarrhythmias. These effects
were abolished by TRPA1KO and attenuated by βAR-inhibition, indicating
TRPA1 as primary and βAR as partial mediators of PG:VG’s immediate cardiac
effects. After exposure, PG:VG increased HR, decreased HRV, and prolonged
QT interval in WT (P<0.05 vs. Air). KO did not significantly alter the post-ex-
posure effects on QT; however, post-exposure effects on HR and HRV were
attenuated by TRPA1KO, and declines in HRV were further inhibited by βAR
blockade, with equivalent impacts of β-inhibition between genotypes. Thus,
e-cig aerosols induce bradycardia during exposure and sympathetic domi-
nance after exposure in mice, and concomitantly impede cardiac repolariza-
tion. E-cig solvent aerosols may increase cardiac risk by inducing autonomic
immunology and arrhythmia via both TRPA1 and βARs in independent but com-
plementary pathways. Additional studies in both animals and humans are
needed to assess the role of these pathways in the effects of chronic exposure
and determine which e-cig aerosol constituents mediate these effects. Our
findings suggest health agencies may reduce harm by regulating e-cig char-
acteristics to diminish byproducts known to stimulate TRPA1.
Acetaldehyde (AA) is an agent that causes an inflammatory reaction; however, the mechanism behind the immune activation by AA is not well understood. Physiologically, it has been shown that 1,4-dihydropyridine-type malondialdehyde (MDA)-AA-lysine (M2AA) adducts are formed by a reaction between AA and the aldehydes of MDA and lysine. We successfully conjugated purified M2AA (pm2AA) to bovine serum albumin (BSA), and we found that pm2AA-BSA is immunogenic in mice. Using our pm2AA-BSA ELISA assay, we detected natural antibodies against M2AA in the blood of mice, and their titers are elevated in atherosclerosis-prone ApoE ko mice. As one of the components of M2AA, MDA is not only one of the most abundant carbonyl compounds caused by lipid peroxidation, but it also possesses unique reactive properties. In addition to its dialdehyde structure, the central carbon of MDA reacts with carbonyl moieties; therefore, MDA can produce various complex lysine adducts with AA. As such, we hypothesized that the combination of AA, MDA, and lysine could lead to the formation of additional immunogenic adducts other than M2AA. In the present study, the reaction product of AA, MDA, and 6-aminoacapric acid (6-ACA, a lysine analog) was fractionated by HPLC. All of the fractions were conjugated with BSA, and each fraction-BSA conjugate was utilized for an ELISA assay to detect antibodies against each fraction product. The immunogenicity of the immunoreactive fractions specific to 1,4-dihydropyridine-type adducts showed a positive response to the fraction containing M2AA, but not to other fractions. Interestingly, when we ran blood samples from wild-type and ApoE ko mice on the ELISA, we found that the titers of antibodies against all of the fractionated products were increased in the blood of ApoE ko mice, indicating the existence of multiple immunogenic epitopes in the reactant of MDA, FA, and 6ACA. Further, we identified that the most significant increase in antibody titer in the blood of ApoE ko mice was not the fraction containing the M2AA adduct. From this, we believe that evaluating serum titers of natural antibodies against various complex MDA-AA-lysine adducts could be used as a biomarker for metabolic syndromes. Our next step is to apply this ELISA assay to human samples obtained from individuals with different LDL levels.

Tyrosine kinase inhibitors (TKI) have greatly improved the treatment and prognosis for a wide range of cancers. Unfortunately, numerous TKIs produce cardiotoxic effects, which were not well predicted during preclinical studies. We developed an in vitro assay, Cardio quickPredict, for predicting cardio-vascular activity based on changes in human iPSC-derived cardiomyocytes (iPSC-CM) metabolism and cell viability, which identifies both functional and structural cardiotoxicants. The assay’s prediction model (PM) is based on the response of three metabolite ratios (viability (VIA)/lactate (LAC), thymidine (THY)/arachidonic acid (ARA), and N-acetylsapartate (NAA)/2-deoxyctydine (2DC)) and predicts the concentration a compound exhibits cardiotoxicity potential. The PM classified 78 compounds with known cardiotoxicity outcomes (49 cardiotoxic, 29 non-cardiotoxic) with 87% accuracy, 90% sensitivity, and 83% specificity. The current study evaluated the utility of this assay for evaluating the cardiotoxicity potential of TKIs. We tested 10 TKIs that induce a variety of cardiotoxic effects, including eight compounds clinically associated with cardiotoxicity (crizotinib, dasatinib, imatinib, lapatinib, nilotinib, sorafenib, sunitinib, and vandetanib) and two compounds considered to be relatively cardiac-safe (axitinib and erlotinib) to compare changes in metabolism of the PM ratios. Human iPSC-CMs were exposed to eight concentrations of each compound for 72 hours and cell viability and metabolites in the spent media were analyzed. Every compound except axitinib altered at least one metabolite independent of a change in cell viability. Crizotinib, dasatinib, erlotinib, imatinib, sorafenib, sunitinib, and vandetanib elicited a response in all three ratios; however, a difference was observed in which ratio was impacted at the lowest concentration. For example, crizotinib altered VIA/LAC at significantly lower concentrations (2-fold) than where a response was observed in NAA/2DC and THY/ARA. In contrast, sorafenib elicited a response in all three ratios at similar concentrations. These results suggest that the Cardio quickPredict assay can be used to screen for cardiotoxicity potential of TKIs and can identify TKIs that cause a range of functional and structural cardiotoxic effects. The metabolic readout of this assay can be combined with other assays, such as the CIPA myocyte assay, to provide a more comprehensive evaluation of a compound’s cardiovascular liability.

The use of microelectrode array technology (MEA) has proven to be a very powerful tool for prediction of proarrhythmic liabilities in iPSC derived cardiomyocytes. This work has focused primarily on the prominent cardiac ion channels with an emphasis on hERG. To this end, the Comprehensive In Vivo Proarrhythmia assay (CIPA) initiative was established and has been tasked with prediction of proarrhythmic compounds in an in vitro setting with acute short term dosing. Although most of the focus on the early work has been on arrhythmias driven primarily by hERG block, the real power of iPSC-derived cardiomyocytes on an MEA platform is predicting unexpected liabilities and long term chronic effects in vitro. These type of studies are currently performed in telemerized dogs at great expense. Here we show examples of compounds whose liabilities were identified with chronic dosing of compounds in an MEA assay. Due to the ability to measure and maintain cells over long periods of time, this platform is ideal for evaluating short and long term exposures early in drug development. Examples of hERG trafficking effects are demonstrated at 24 and 48 hours utilizing Pentamidine. The FPD is significantly lengthened while the amplitude of the repolarization peak is reduced. The hepatitis C drug BMS-986094, which failed in clinical trials, is shown to progressively deteriorate cardiomyocyte health as compared to safe hepatitis C drugs such as sofosbuvir in 14-day studies. The results show a complete loss of beating at 400mV and above while showing significant effects on the electrophysiology as low as 80mV. We also tested compounds that have other unexpected responses such as delayed onset arrhythmias and Na amplitude effects that are not observed acutely and cannot be predicted by channel screening. These compounds confirmed results found in animal studies. These results demonstrate that iPSC derived cardiomyocytes on an MEA platform are an effective tool for screening compounds to identify unexpected long term liabilities before expensive preclinical animal experiments are performed, thus improving the chances of moving forward with a safe compound.

Tyrosine kinase inhibitors (TKI) have greatly improved the treatment and prognosis for a wide range of cancers. Unfortunately, numerous TKIs produce cardiotoxic effects, which were not well predicted during preclinical studies. We developed an in vitro assay, Cardio quickPredict, for predicting cardio-vascular activity based on changes in human iPSC-derived cardiomyocytes (iPSC-CM) metabolism and cell viability, which identifies both functional and structural cardiotoxicants. The assay’s prediction model (PM) is based on the response of three metabolite ratios (viability (VIA)/lactate (LAC), thymidine (THY)/arachidonic acid (ARA), and N-acetylsapartate (NAA)/2-deoxyctydine (2DC)) and predicts the concentration a compound exhibits cardiotoxicity potential. The PM classified 78 compounds with known cardiotoxicity outcomes (49 cardiotoxic, 29 non-cardiotoxic) with 87% accuracy, 90% sensitivity, and 83% specificity. The current study evaluated the utility of this assay for evaluating the cardiotoxicity potential of TKIs. We tested 10 TKIs that induce a variety of cardiotoxic effects, including eight compounds clinically associated with cardiotoxicity (crizotinib, dasatinib, imatinib, lapatinib, nilotinib, sorafenib, sunitinib, and vandetanib) and two compounds considered to be relatively cardiac-safe (axitinib and erlotinib) to compare changes in metabolism of the PM ratios. Human iPSC-CMs were exposed to eight concentrations of each compound for 72 hours and cell viability and metabolites in the spent media were analyzed. Every compound except axitinib altered at least one metabolite independent of a change in cell viability. Crizotinib, dasatinib, erlotinib, imatinib, sorafenib, sunitinib, and vandetanib elicited a response in all three ratios; however, a difference was observed in which ratio was impacted at the lowest concentration. For example, crizotinib altered VIA/LAC at significantly lower concentrations (2-fold) than where a response was observed in NAA/2DC and THY/ARA. In contrast, sorafenib elicited a response in all three ratios at similar concentrations. These results suggest that the Cardio quickPredict assay can be used to screen for cardiotoxicity potential of TKIs and can identify TKIs that cause a range of functional and structural cardiotoxic effects. The metabolic readout of this assay can be combined with other assays, such as the CIPA myocyte assay, to provide a more comprehensive evaluation of a compound’s cardiovascular liability.

The use of microelectrode array technology (MEA) has proven to be a very powerful tool for prediction of proarrhythmic liabilities in iPSC derived cardiomyocytes. This work has focused primarily on the prominent cardiac ion channels with an emphasis on hERG. To this end, the Comprehensive In Vivo Proarrhythmia assay (CIPA) initiative was established and has been tasked with prediction of proarrhythmic compounds in an in vitro setting with acute short term dosing. Although most of the focus on the early work has been on arrhythmias driven primarily by hERG block, the real power of iPSC-derived cardiomyocytes on an MEA platform is predicting unexpected liabilities and long term chronic effects in vitro. These type of studies are currently performed in telemerized dogs at great expense. Here we show examples of compounds whose liabilities were identified with chronic dosing of compounds in an MEA assay. Due to the ability to measure and maintain cells over long periods of time, this platform is ideal for evaluating short and long term exposures early in drug development. Examples of hERG trafficking effects are demonstrated at 24 and 48 hours utilizing Pentamidine. The FPD is significantly lengthened while the amplitude of the repolarization peak is reduced. The hepatitis C drug BMS-986094, which failed in clinical trials, is shown to progressively deteriorate cardiomyocyte health as compared to safe hepatitis C drugs such as sofosbuvir in 14-day studies. The results show a complete loss of beating at 400mV and above while showing significant effects on the electrophysiology as low as 80mV. We also tested compounds that have other unexpected responses such as delayed onset arrhythmias and Na amplitude effects that are not observed acutely and cannot be predicted by channel screening. These compounds confirmed results found in animal studies. These results demonstrate that iPSC derived cardiomyocytes on an MEA platform are an effective tool for screening compounds to identify unexpected long term liabilities before expensive preclinical animal experiments are performed, thus improving the chances of moving forward with a safe compound.
Effect of TSN3015 on the Development of Pulmonary Arterial Hypertension in Male Sprague-Dawley Rats

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TSN3015 (β-naphthoflavone), an AhR agonist with seborepressive activity, was used as an anti-acne medication. In toxicology studies in rats, microscopic changes suggestive of pulmonary arterial hypertension (PAH) were observed in the lungs and hearts of some TSN3015-treated animals. This study was undertaken to determine the effect of TSN3015 on pulmonary artery pressure (PAP). Conscious pulmonary artery-telemeterized male Sprague-Dawley rats were subcutaneously administered either vehicle or TSN3015 at 3 mg/kg for 28 consecutive days, followed by a 55-day recovery period. Clinical observations, telemetry data and blood samples were collected at defined time points throughout the study. The heart and lungs were collected at necropsy for weights and histological examination. No adverse clinical observations were noted over the 84-day study though 2 animals died during the recovery period. A 3 mg/kg dose of TSN3015 elicited an increase in systolic PAP that remained elevated for up to 49 days after the last dose was administered (36.2±8.3 mmHg on Day 84 vs 31.5±2.6 mmHg on Day -1), with the peak increase occurring on Day 49 (range: +34.3% to +117.8%).

TSN3015 did not notably affect the diastolic PAP or heart rate. Necropsy findings showed an increase in right heart weight and Fulton Index (FI) in animals given TSN3015, while histological examinations showed microscopic changes in the lungs and hearts of some TSN3015-treated animals. Microscopic findings in the lung consisted of minimal hypertrophy/hyperplasia of the tunica media of medium- and small-size arteries and minimal foamy alveolar macrophages; the heart findings presented as microscopic alterations predominately in the right ventricle. TSN3015-treated animals with lung pathology had a statistically significant elevation in sPAP and FI when compared to either vehicle-treated animals (p<.001 for both parameters) or TSN3015-treated animals without lung pathology (p<.001 for sPAP; p=.004 for FI). Overall, 9 of the 12 animals treated with TSN3015 developed right heart hypertrophy as indicated by an increased FI (0.373±0.016 TSN-treated animals vs 0.318±0.015 for vehicle-treated animals, p<.05), and these elevated FI values correlated with histologic findings in the heart and lungs. Thus, under the confines of this study, TSN3015 appears to create characteristics consistent with PAH in male rats.

The ability to generate a reliable VM lead from the 3 input leads in NHP and produced expected changes in QTc and JTp each of the drug combinations.

Effect of TSN3015 on the Development of Pulmonary Arterial Hypertension in Male Sprague-Dawley Rats

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Qtc was identified as a central proarrhythmic risk biomarker in ICH S7A but recent clinical data have revealed limitations when using QTC mostly related to false positives. Drugs with balanced multi-ion channel inhibition were shown to prolong QTC without increased proarrhythmic risks. Research led by the ICH FDA has identified JTPc as a reliable ECG biomarker that evaluates drug-induced proarrhythmic risk in clinical trials and HESI showed similar effects in canines and non-human primates. In humans or non-clinical species, proarrhythmic drugs are associated with an increase in QT but also JTPc. The current study evaluated the effects of common ECG confounders on QTc, JTpca and Tpeca. Beagle dogs were anesthetized with isoflurane and subjected to progressive hyperthermia (42°C), hypothermia (33°C) (n=4) or epinephrine IV injections (0.03 mg/kg) (n=10).

Dofetilide, verapamil and ranolazine were used as positive control drugs. All ECG parameters (QTc, JTpca and Tpeca) were subjected to individual rate correction. QTc (slope -9.2 mseg/degree Celsius) and JTpca (-6.7 mseg/degree Celsius) durations were negatively correlated with core body temperature but Tpeca was minimally affected. Epinephrine was associated with QTc and JTpca shortening which could be related to under-correction in the presence of tachycardia but minimal effects on Tpeca.

Dofetilide was associated with significant increase in JTpca while verapamil and ranolazine tended to prolong Tpeca. Multi-lead derived ECGs showed improved morphology for T-peak detection. The above results highlight the importance of potential confounders on the traditional ECG biomarker QTc but also on JTpca and Tpeca. These potential confounding effects need to be considered in the interpretation of ECG biomarkers during proarrhythmic risk assessment in non-clinical drug development.

Discrimination of the Effects of Multi-Channel Ion Blockade Using ECG Biomarkers in St. Kitts Green Monkeys

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FDA and the HESI JTP working group have laid the groundwork for use of J to T-pea (JTP) interval to differentiate drugs that exhibit multichannel block in clinical and preclinical studies. Clinical studies typically include 12-lead electrocardiography (ECG) monitoring with JTP assessed from a vector magnitude (VM) lead while preclinical assessment is often based on a single ECG vector such as Lead II (L2). In this study we usedStellar multichannel ECG, blood pressure, temperature, and activity telemetry implants (TSE Systems) to assess changes in corrected QT interval (QTc) and JTPc intervals for human ether-a-go-go related gene (hERG) potassium channel blockers (Dofetilide; Dof) in conjunction with calcium (Verapamil; Ver) or sodium (Mexiletine; Mex) ion channel blockers using both VM and L2. St. Kitts green monkeys (n=4) were instrumented with subcutaneous electrodes to record limb leads I, II, and III. ECGs were analyzed with the Rhythm Express software (VivaQuant) to remove noise, assess arrhythmias, and derive interval measurements from L2 and VM leads. This study was undertaken to determine the importance of potential confounders on the traditional ECG biomarker QTc but also on JTPca and Tpeca. These potential confounding effects need to be considered in the interpretation of ECG biomarkers during proarrhythmic risk assessment in non-clinical drug development.

An Integrative Lipidomic and Proteomic Approach to Understanding the Nanoparticle-Biocorona

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When nanoparticles (NPs) enter a physiological environment, a biocorona (BC) forms on the surface, impacting both NP properties and cellular interactions, leading to pharmacological and toxicological consequences. The majority of NP-BC research has focused on the protein components of the BC, while other components such as lipids lack sufficient evaluation. Our current evaluation utilized an integrated -omics approach (lipidomics and proteomics) to examine the identities and quantities of lipids as well as proteins that adsorb to the NP surface. We hypothesized that gold nanoparticles (AuNPs) would form BCs with distinct protein and lipid content based on physicochemical properties of NPs (size and coating) and disease states (healthy or obese). AuNPs with two different coatings (PVP or citrate) and diameters (20 nm or 100 nm) were incubated individually in either pooled healthy or obese human serum to form a BC. Lipids were extracted from the resultant BC via the Bligh-Dyer method, while peptides were isolated by digestion of proteins and subsequent column separation. AuNP size and zeta potential were found to be differentially altered following incubation in serum samples. Analysis of both proteins and lipids demonstrated that the biomolecular profiles of AuNP-BCs incubated in normal serum tend to be more unique than those incubated in obese serum, and that citrate-coated AuNPs form more similar BCs between serum conditions than PVP-coated AuNPs. Additionally, in normal serum, BC composition is influenced primarily by AuNP coating as opposed to size. Conversely, in obese serum size is the primary property determining AuNP-BC formation. Lipid classes were found to differentially adsorb to the NP surface. Specifically, the most abundant class of lipids in the BC was glycerolipids, with 300 unique species identified in each NP-BC. The least abundant classes were sulfatides and glycosylinositolphosphoryl ceramides, with less than three of each unique species identified in each NP-BC. The most abundant class of lipids in the BC was glycerolipids, with over 300 unique species identified in each NP-BC. The least abundant classes were sulfatides and glycosylinositolphosphoryl ceramides, with less than three of each unique species identified in each NP-BC. The most abundant class of lipids in the BC was glycerolipids, with over 300 unique species identified in each NP-BC. The least abundant classes were sulfatides and glycosylinositolphosphoryl ceramides, with less than three of each unique species identified in each NP-BC.
The liver and the mononuclear phagocyte system are a frequent target for engineered nanomaterials, either as a result of particle uptake and spread from primary exposure sites or systemic administration of therapeutic and imaging nanoparticles. In this study, we performed a comparative analysis of the toxicological impact of 29 metal oxide nanoparticles (NPs), some commonly used in consumer products, in transformed or primary Kupffer cells (KCs) and hepatocytes. We not only observed differences between KCs and hepatocytes, but also differences in the toxicological profiles of transition-metal oxides (TMOs, e.g., Co3O4) versus rare-earth oxide (REO) NPs (e.g., Gd2O3). While pro-oxidative TMOs induced the activation of caspases 3 and 7, resulting in apoptotic cell death in both cell types, REOs induced lysosomal damage, NLRP3 inflammasome activation, caspase 1 activation, and pyroptosis in KCs. Pyroptosis was accompanied by cell swelling, membrane blebbing, IL-1β release, and increased membrane permeability, which could be reversed by knockdown of the pore forming protein, gasdermin D. Though similar features were not seen in hepatocytes, the investigation of the cytotoxic effects of REO NPs could also be seen to affect macrophage cell lines such as J774A.1 and RAW 264.7 cells as well as bone marrow-derived macrophages. These phagocytic cell types also demonstrated features of pyroptosis and increased IL-1β production. Collectively, these findings demonstrate important mechanistic considerations that can be used for safety evaluation of metal oxides, including commercial products that are developed from these materials.

### 3290 Transcriptional Signatures in Human LP9 Mesothelial Cells after Treatment with Differentially Purified and Surface Functionalized Multiwalled Carbon Nanotubes

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Exposure to certain long and straight multiwalled carbon nanotubes (MWCNT) has been implicated in adverse biological effects, including induction of malignant mesothelioma. Besides morphology, functionalization seems to affect the toxic potential of MWCNT. The ERA-ETN SiINN project ICONS (“International Collaboration On Nanotube Safety”) therefore aimed at mechanistically evaluating and ranking the pro-fibrotic and genotoxic potential of seven differentially purified (chemically or thermally) and functionalized (TMOs, e.g., Co3O4) versus rare-earth oxide (REO) NPs, made from one batch of industrially relevant Nanocyl NC7000 (tangled morphology). As a prerequisite, initial tests indicated sterility and lack of relevant endotoxin contamination of the materials. In a subproject performed by Fraunhofer ITEM, human peritoneal mesothelial LP9 cells (target cells for mesothelioma development) were incubated for 24 h with 5 μg/cm2 of the diverse MWCNT, followed by microarray analysis to evaluate material-specific transcriptional signatures. In subsequent bioinformatic analyses, significant number of differentially-regulated genes (DEGs) was noted for all eight MWCNT (range: 555 to 2524 genes), with the amount of differentially-regulated genes and the observed fold-change values being strongly dependent on the MWCNT type. Interestingly, number of DEGs was about two- to five-fold higher for the surface-modified samples than for pristine/unmodified ENPs.

### 3291 Induction of Oxidative DNA Damage and Promotion of Epithelial Mesenchymal Transitions through Emitted Engineered Nanoparticle Exposure in Human Primary Small Airway Epithelial Cells

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Engineered metal nanoparticles (ENPs) are frequently incorporated into aero- solized consumer products, known as nano-enabled products (NEPs). There is a growing concern regarding consumer pulmonary exposures as certain NEPs produce high concentrations of emitted ENPs that are chemically modified by constituent chemicals. While understanding the potential health effects of pristine/unmodified ENPs has gained importance, a significant knowledge gap still exists surrounding emitted ENP biological activity and potential respiratory effects. Our research evaluated emitted ENP induced oxidative stress and 8-oxoguanine, a prominent base lesion, repaired by DNA glycosylase OGG1 in the Base Excision Repair (BER) pathway and how these outcomes can contribute to mesothelial mesenchymal transition (EMT) - a precursor to airway remodeling. Our main hypothesis is chemically modified ENPs emitted from NEPs increase oxidative stress levels, resulting in oxidative DNA damage and EMT through OGG1-BER pathways. In this study, we utilized an automated NEP generation system to monitor and gravimetrically collect aerosols from two aerosolized NEP cosmetic lines to conduct physico-chemical, colloidal, and toxicological assessments. Scanning electron microscopy (SEM-EDX) and dynamic light scattering (DLS) were used for colloidal characterization. The toxicological profiles of pristine and emitted nanoparticles were compared using human primary small airway epithelial cells to assess ROS and oxidative stress by a recently developed assay. Single stranded DNA damage and 8-oxoguanine were detected using the CometChip® assay after 24-hour exposure. EMT was assessed via Western blot analysis on lysates from 21-day exposures to evaluate modulation of E-cadherin and Vimentin. SEM-EDX analysis revealed that emitted particles were spherical and primarily titanium dioxide or iron oxide. DLS assessments indicated that certain emitted ENPs were more negatively charged than pristine ENPs. Toxicity results indicated higher oxidative stress at 24-hour exposure due to emitted nanoparticles in comparison to pristine ENPs along with increased levels of single-stranded DNA damage and 8-oxoguanine. Western blot analysis revealed Vimentin induction after 21-day exposure indicative of EMT. This work elucidates the role oxidative DNA damage plays in potential pulmonary health risks due to NEP exposures and thus the importance of regulation and consumer product safety.
of pleural mesothelioma replicating the pathogenesis of human disease and highlighting commonality in the hazard mechanism of long pathogenic fibers at the molecular level with epigenetic changes preceding malignant transformation of inflammatory lesions. Crucially, our findings reinforce concerns that long straight CNT may pose an asbesto-like hazard, leading to mesothelioma.

### 3293 An In Vitro Approach to Assess the Pulmonary Fibrosis of Nanomaterials

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Pulmonary fibrosis is one of the main adverse health effects that may be linked to the inhalation of multi-walled carbon nanotubes (MWCNTs). Several jurisdictions require a 90-day rodent test to assess the inhalation toxicity of substances of concern. Ethical, scientific, and financial concerns related to in vivo testing have led to an increase in the use of animal-free approaches. Presented here is an approach informed by a mechanistic understanding of fibrosis, which uses a primary human cell-based system (EpiAlveolarTM) exposed to MWCNTs at realistic exposure concentrations. EpiAlveolarTM (MatTek Corp.) - a reconstructed primary human alveolar tissue model comprised of alveolar epithelial cells, pulmonary endothelial cells, and fibroblasts, with or without macrophages (THP-1 cells) - was characterized to assess cell morphology, barrier properties, cytotoxicity, and ability to predict fibrosis in response to a positive control chemical (transforming growth factor-beta (TGF-β)). EpiAlveolarTM was then exposed to MWCNTs at the air-liquid interface (using the VITROCELL® Cloud system) for up to 21 days to assess their fibrotic potential using immunostaining, transepithelial electrical resistance (TEER), and collagen 1A1 and fibronectin release. MWCNTs (Mitsui 7 or Nanocyl) were characterized using a combination of qualitative and quantitative methods to assess the nanomaterial form and concentration that reaches the cell layer, including ultraviolet-visible-near infrared spectroscopy, bright field and enhanced dark field microscopy, and quartz crystal microbalance. The goal of this work is to develop an in vitro testing strategy using human-relevant methods to predict pulmonary toxicity and to enable effective risk assessment of substances including MWCNTs.

### 3294 A Novel Method of Measuring ENM Induced Lipid Disruption in Macrophages and Model Membranes Systems


The expanded use of nanotechnology has led to increased production and use of engineered nanomaterials (ENM), resulting in an increased risk of human exposure. Many ENM are taken up by cells where the ENM accumulate in phagolysosomes. Some ENM have been shown to cause a leakage of phagolysosomes, resulting in phagolysosomal membrane permeability (LMP). LMP leads to the release of proteolytic enzymes into the cytosol, leading to release of inflammatory cytokines and cytotoxicity. This suggests that ENM may interact directly with the lipid membrane, disrupting the normal state. Time-resolved fluorescence anisotropy measurements, using suitable lipid probes, can measure changes in membrane characteristics, such as lipid order (Lo) and disorder (Ld). These changes are quantified as a cone angle, the free movement of the probe in the membrane. Additionally, THP-1 cells stably expressing a YFP-ASC protein can be used to determine inflammasome formation, an immediate downstream event of LMP. In this work, THP-1 cells and 100 nm liposomes made of POPC (1-palmitoyl-2-oleoyl-glycerol-3-phosphocholine) and DOPS (1,2-dioleoyl-sn-glycero-3-phospho-L-serine) were used as model systems for interaction with ENM. Both models were exposed to 12.5 to 100 µg/ml titanium dioxide (TiO2) at 210 nm ZnO nanoparticles. Fluorescence membrane probe DI-4-ANEPPDHQ and a time-resolved fluorometer were used to determine the changes in lipid Lo/Ld of the liposomes. Inflammasome formation (specks) were quantified using a laser scanning cytometer. 2.5-3.5% of control THP-1 cells at both 24 and 4 h speck formation, indicating that some cells had spontaneous speck formation. THP-1 cells exposed to 100 µg/ml TiO2 for 4 h had a significant increase in speck formation. 11.4% after 24 h the 50 µg/ml TiO2 increased to nearly the same level as the 4 hr 100 µg/ml. There was no significant increase in speck formation at 100 µg/ml TiO2 between 4 and 24 h. ZnO exposed THP-1 had a significant increase in speck formation at 50 µg/ml after 4 h (14%) and at 25 and 50 µg/ml after 24 h, 25% and 45%, respectively. POPC (neutral charge) liposomes exposed to 100 µg/ml TiO2 had a decrease in cone angle from 38° to 29°, with no significant change in DOPS (negative charge) liposomes. ZnO exposure (100 µg/ml) saw a decrease from 36° to 26°, with no change in POPC. This indicates that while both materials may be disrupting lipid membranes, the target lipids are different. Funding: NSF MRI CHE-1531520, NIH R01ES023209, 1F32ES027324, P20GM103546 and P03GM103338.

### 3295 Developing an Animal Model of Drug-Induced Respiratory Depression to Assess Co-Administration of an Opioid with Other Sedative Psychotropic Drugs


Opioids and benzodiazepines are frequently co-prescribed to patients with both pain and psychiatric or neurological disorders. Co-prescription of these drugs increases the risk for severe respiratory depression and death. In 2016, the US Food and Drug Administration required the addition of boxed warnings describing this risk to labeling for all prescription opioids and benzo- diazepines. Other sedating psychotropic drugs (SPDs) with differing mechanisms of action (e.g., antipsychotics, antidepressants, non-benzodiazepine sedative-hypnotics, etc.) may be increasingly prescribed in place of benzodiazepines. Despite being marketed for years, many SPDs have neither human nor animal data to quantify or qualify the potential for causing respiratory depression, either alone or in combination with an opioid. In this work, an animal model was established to address this question and generate data to inform regulatory decision-making. Arterial partial pressure of oxygen (pO2) and carbon dioxide (pCO2) were selected as practical and sensitive measures of respiratory depression. An oral exposure route was selected for all drugs to allow for clinical use. Diazepam was selected as a benzodiazepine positive control to demonstrate that the model was sufficiently sensitive to detect an additive or synergistic effect on respiratory depression with the opioid, oxycodone. Pharmacokinetic studies were conducted at three dosing concentrations with oxycodone (opioid; 6.25, 65, 150 mg/kg) and diazepam (benzodiazepine; 2, 20, 200 mg/kg) to determine whether oxycodone and diazepam caused similar pO2 and pCO2 changes at different combinations (200 mg/kg). Based on area under the curve assessment, oxycodone 150 mg/kg and diazepam 20 mg/kg were approximate clinical single dose exposures in humans. Diazepam was administered 30 minutes after oxycodone to deliver peak serum concentrations of both drugs at near the same time. Decreases in pO2 and increases in pCO2 consistent with additional respiratory depression were observed in rats co-administered oxycodone and diazepam compared to oxycodone alone. These findings support the utility of this animal model for assessing opioid-induced respiratory depression and its potential exacerbation by other SPDs.

### 3296 Thalidomide Inhibits Human iPSC Mesendoderm Differentiation by Modulating Cereblon-Dependent Degradation of SALL4

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Thalidomide exposure during a narrow time window of fetal development produces phocomelia in humans. Several teratogenicity mechanisms for thalidomide have been proposed, but recent evidence suggests thalidomide exerts teratogenicity through binding to and modifying the substrate-binding specificity of cereblon, a component of the ubiquitin ligase complex with DDB1 and CUL4A. Thalidomide enhances cereblon-dependent degradation of the transcription factor SALL4, mutations in which are known to produce developmental defects in humans and mice that phenocopy thalidomide-induced limb defects in humans. We interrogated thalidomide-induced degradation of SALL4 as a possible mechanism for limb teratogenicity using human induced pluripotent stem cells (hiPSCs). Thalidomide inhibited hiPSC definitive endoderm differentiation to a SOX17+ phenotype (IC50 = 8 µM), resistant to thalidomide-mediated interference of endoderm differentiation, suggesting the effect of thalidomide on differentiation was dependent on cereblon binding and SALL4 degradation. We further sought to explore the influence of thalidomide on hiPSC differentiation to lateral plate mesoderm, which is the developmental origin of the ENM tested. Mesendodermal differentiation was up-regulated by thalidomide. Cereblon (CBL) and SALL4 expression was decreased in thalidomide-treated EpiAlveolar (T) expression and after 48 h induced GATA4, FOXF1, and PITX1 expression and reduced NANG and POU5F1 expression in hiPSCs consistent with
Impact of Monoclonal Antibody (MoAb), Payload and Linker Structure in Antibody Drug Conjugates (ADCs) on Primitive Myeloid Progenitors (CFU-GM), or Mature Neutrophil Cytotoxicity

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ADC design involves a tumor antigen-specific antibody, a linker and a cytotoxic drug. In some clinical trials, ADCs caused neutropenia in patients despite having targets that are not present on hematopoietic stem cells (HSC) or neutrophils. The CFU-GM assay, predictive of clinical neutropenia and validated by Pessina et al. (2003), evaluated compound effects on primitive myeloid precursors, whereas the platform described by Zhao et al. (2017) suggested the extracellular cleavage of the ADC linker was responsible for cytotoxicity to differentiating mature neutrophils. We tested 2 naked antibodies, 1 cytotoxic drug and multiple ADCs with different cleavable linker sites (valine-alanine, valine-citriulline and maleimidomethyl cyclohexane-1-carboxylylate) on both platforms to compare their utility to identify potential off-target bone marrow (or hematologic) effects of ADCs. For the CFU-GM assay, compounds were added to cells in a liquid medium for 72 hours, then the treated cells were transferred to a methylcellulose-based medium for 14 days. To assess neutrophil toxicity using the Zhao platform, CD34+ cells were cultured for 3 days initially to expand the HSC followed by 4 days in a differentiation media and then another 4 days with various cytokines. The various MAbPs, payload and ADCs were added to the culture for the final 6 days (G-CSF only) and assessed for CD15. The naked antibodies, IC1-Maia and trastuzumab, had no effect on CFU-GM or neutrophil numbers. The payload (drug plus linker) demonstrated significant toxicity to the CFU-GM (IC50 of 0.0060 nM), but much less potency to mature neutrophils (IC50 > 0.3 nM). Kadcyla (FDA approved trastuzumab-based ADC) was 3 times more inhibitory to the CFU-GM assay compared to mature neutrophils, whereas trastuzumab-vc-MMAE ADC was 5 times more inhibitory to mature neutrophils compared to primitive progenitors. There was more than a 10-fold difference in the IC50 values of the ADCs R347-Maia-SG3294, CS1-Maia-SG3294 and 1C1-Maia-SG3249 between assays, with more toxicity towards the CFU-GM than the differentiating neutrophils. With many ADCs in clinical trials, these assay platforms may be useful for predicting preclinical development to identify potential off-target effects of antibodies and payloads or susceptibility for single or dual cleavage sites to release the cytotoxic drug.

Bilateral Distal Femoral Epiphyseal Defect Models for Safety Testing: A 5-Week Rat Bone Healing Study

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Low trauma fractures represent clinical complications from our aging society. Most fractional injuries are known to occur in long bones with inevitable low trauma fractures representing 30% of all fractures. Per-op and terminal digital radiography was performed on male Sprague-Dawley rats. Following an IACUC-approved protocol from an AAALAC-accredited institution, nine (9) female Sprague-Dawley rats were used to evaluate if 2-mm critical-size defects in distal femoral condyles were adequate for toxicology evaluation of drugs with potential impact on bone remodeling. Under anesthesia, both hindlimbs were prepped and draped in sterile fashion. Then, bilateral cylindrical defects of 2x5mm (DxL) were created in distal femoral condyles. Per-op and terminal digital radiography was performed on all femurs. Then, at 5 weeks post-op, all femoral defect sites were harvested, decalcified, and embedded in paraffin. Central longitudinal histological sections were then taken from the bone defect site and prepared for microscopic examination following H&E and Goldner’s Trichrome staining. Histopathology scoring was performed on the quality of new bone formation, while the extent of new bone formation was quantified by histomorphometry (Table 1). Regardless of treatment, all defect sections demonstrated significant bone remodeling as characterized by the ratio of the cortical bone to the cellular bone (x3). Results indicate that creating a 2-mm long-bone defect enables evaluation of potential drug effects on bone healing in toxicology. In contrast, a 2-mm femoral epiphyseal defect will not be considered a critical-size defect in the femoral condyle (x1), but lacks representative improvement in healing observed from the cortical bone to the cellular bone (x3) for this purpose. When this is met, this rodent femoral defect model may then represent a promising one to evaluate the superiority of new bone substitutes with osteoconductivity and osteoinductivity properties.

Determining Mechanisms of Mitochondrial Toxicity Using Agilent Seahorse XF Technology


Mitochondrial dysfunction is increasingly implicated in the etiology of drug induced toxicities and is a major reason for safety-related compound attrition and post-market drug withdrawals. Most assays currently used for mitochondrial toxicity studies are not direct, not specific and provide limited mechanistic information. Here, a standard workflow is presented to enable a comprehensive characterization of the mechanism of action of mitotoxic compounds. A library of commercially available small-molecule compounds was screened in npsG2 in dose- and time-response studies using the Agilent Seahorse XF analyzers. Performing the XF-Real time ATP Rate Assay that simultaneously measure the mitochondrial and glycolytic activity in intact cells we identified several compounds with mitotoxic activity before observing significant changes in cell viability after either acute (1 hr) treatment or long exposure (18 hr) treatment. Interestingly, we found that acute treatment, the fatty acid amide hydrolase (FAAH) inhibitor PF-3845 induced a transient decrease of mitochondrial ATP production that is almost fully compensated with an increase in glycolytic activity without significant impact in cellular bioenergetics inside the dose range analyzed. Using the XF Mito Stress Test we were able to confirm the inhibition of mitochondrial function under both basal and stressed conditions. Additionally, using the permeabilizing agent XF Plasma Membrane Permeabilizer we were able to identify the mechanism of action of the mitotoxic compound at the molecular level. Treatment of cells for 1 hr with the FAAH inhibitor induced a dose-dependent decrease of complex I activity when respiring on various substrates but did not affect complex II-dependent or complex IV-dependent respiration. The proposed workflow provides a specific and sensitive assay to study mitochondrial function that can be used for deeper evaluation of safety of lead compounds, to discard presence of off-target effects or to characterize the mechanism of mitotoxicity observed in pre-clinical studies.

Occurrence of Spontaneous Arrhythmias in Freely Moving Non-Treated Healthy Telemetered Sprague-Dawley Rats

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The aim of this analysis was to characterize the occurrence of arrhythmias in freely moving non-treated healthy Sprague-Dawley rats. Male (n=55) and female (n=40) Sprague-Dawley rats (297-654g) were implanted with a telemetry transmitter (Data Science International, model HD-511-F2) with ECG in DII. Arrhythmias were assessed from continuous ECG monitoring over a 48-hour period, and data analyzed using an automated platform (Data Insights™ for the Ponemah™ software v6.3). A total of 1825 ventricular ectopic arrhythmias were identified on freely moving Sprague-Dawley rats. Male (n=55) and female (n=40) Sprague-Dawley rats (297-654g) were implanted with a telemetry transmitter (Data Science International, model HD-511-F2) with ECG in DII. Arrhythmias were assessed from continuous ECG monitoring over a 48-hour period, and data analyzed using an automated platform (Data Insights™ for the Ponemah™ software v6.3). A total of 1825 ventricular ectopic arrhythmias were identified on freely moving Sprague-Dawley rats. The occurrence of arrhythmias in Sprague-Dawley rats was observed in pre-clinical studies. This study represents a promising one to evaluate the superiority of new bone substitutes with osteoconductivity and osteoinductivity properties.

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it was noted that individual animals tended to have a predominant ventricular depolarization pattern (i.e., high repetition of a specific ventricular depolarization scheme) possibly originating from a ventricular ori. Premature junctional complexes were more frequent during the dark period and were more frequent in males than females. As expected, 2nd degree atroventricular (AV) blocks, sinus pauses and junctional escape complexes were noted with increased incidence during daytime (i.e., when rats slept). Most AV blocks (57%) were Mobitz type I while 2nd degree AV block Mobitz type II represented 43%. This work may be considered to support interpretation of arrhythmias in rat telemetry studies, by providing a thorough baseline and a better understanding of gender- and time-dependent effects. The prevalence of commonly observed arrhythmias in rats highlights the importance to thoroughly evaluate each animal prior to study initiation.

3301 Application of In Vitro Multi-Parametric Analysis for Early De-Risking Cardiac and Mitochondrial Toxicity of Ciluprevir: A HCV NS5A/4A Serine Protease Inhibitor


Ciluprevir (BILN 2061), an investigational drug inhibiting HCV NS5A/4A serine protease, induced myocardial vacuolization corresponding to mitochondrial swelling and diminished left ventricular cardiac ejection fraction in a 28-day micro dosing mechanistic study. Detection of cytochrome c release was also noted, possibly due to mitochondrial swelling and/or mitochondrial dysfunction due to cardiotoxicity. Hereby, we retrospectively evaluated a multi-parametric assay (MPA) approach to assess cardiac and mitochondrial function in vitro, as tools for identifying risk for ciluprevir toxicity. Ciluprevir showed minor/no effect on cardiac ion channels (hERG, hIKs, or hNav1.5). When studied in human induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CMs), ciluprevir increased both Ca2+ transient and impedance beat duration, and slowed the beating rate concentration-dependently starting at 11μM. It had no effect on Ca2+ transient amplitude, but associated to major, transient (+21%) increases in impedance amplitude. Ciluprevir 7-day treatment assays showed mitochondrial respiration at 100 μM and high-content imaging assay revealed that it affected mitochondrial intensity and texture, cytoplasmic and nuclear features at IC50s (50-65.4 μM) in hiPSC-CMs. While ciluprevir tested negative using HepG2 glucose/galactose medium switch assay, it acutely induced proton leak and showed an uncoupler-like profile (60 and 100 μM) while had no effect on electron transport chain activity up to 100 μM. The increased glycolysis is likely related to the metabolic adaptation to mitochondrial inhibition. In conclusion, MPA approach supports cardiac and mitochondrial disruption caused by ciluprevir. Uncoupler-like property corroborates with mitochondrial swelling observed in rhesus monkey heart tissues. Furthermore, this retrospective case study demonstrated the unique value of combining acute and long-term in vitro MPA approach as a tool for early de-risking cardiac and mitochondrial safety liability of new therapeutic drug candidates and facilitating preclinical candidate selection.

3302 Characterization of EEG Morphologies during Drug-Induced Seizures and Peri-Ictal Changes in Nonclinical Species


Drug effects on hemostasis may represent intended pharmacology or can be an observed adverse effect. Bleeding time tests (BTT) are used for diagnosis in patients, but also to assess platelet function in non-clinical drug safety assessments. The goal of the study was to assess and compare anatomical sites for BTT in species commonly used for non-clinical drug development. Beagle dogs, cynomolgus monkeys, New Zealand White rabbits and Sprague-Dawley rats were used to assess BTTs at several anatomical regions (i.e., gums, cranium, upper lip, pinna, inner cheek). A single use Surgicutt® bleeding time device for consistency in incision length and depth (1 mm depth by 5mm length) was employed. Craniun presented low inter-occasion variability in all species, most likely due to cutaneous attachment to the cranial structures which enables stable application of Surgicutt® devices. Gums and pinna (evaluated in dogs, monkeys and rabbits only) presented intermediate variability. Lips showed greater variability, possibly due to differences in tissue immobilization and/or saliva levels. Between species, monkey lips (lower lip 10.9 min. and upper lip 7.5 min.) presented the highest inter-occasion variability, but cranium (5.4 min.), pinna (5.4 min.) and inner cheek (4.7 min.) showed values within expected ranges. These results support the use of BTT assessments in duplicate or triplicate at each time point to increase sensitivity of this assay in the context of toxicology assessments.

3303 Safety Risks Due to Low Seizure Detection Specificity: Applications and Limitations of Automated EEG Algorithms in Preclinical Studies

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The preclinical seizure liability of investigational compounds frequently involves EEG assessments including the presence of compound-induced electrographic seizures and preictal epileptiform morphologies indicative of focal events (e.g., sharp waves). The EEG is a clinically-translatable signal and remains the gold standard for sensitive assessment of a drug’s seizure liability. With the widespread use of affordable video-telemetry EEG, it is presently possible to collect vast amounts of data spanning from 24 hours to many weeks, thus shifting the challenges from data collection to quality data analysis. Most preclinical EEG visualization platforms offer tools intended to automate the search for salient features of the signal related to seizure risk, based usually on a variation of amplitude and frequency or spike train detection. Similar tools are used in the clinic supporting the neurologist-driven EEG interpretation, which remains the only acceptable diagnostic methodology due to the complexity and variability of the signal. Here we present case studies using EEG from rodents, dogs and monkeys, illustrating a consistent lack of specificity of automated algorithms resulting in false negative and false positive findings. Recommendations are made for a tiered approach to preclinical EEG data analysis, including expert-driven and computerized tool-box aided approaches, that provides a sensitive and systematic analysis that reduces the risk of incorrect interpretation and omissions and increases the value of this information-rich functional measure.

3304 Evaluation of Primary Hemostasis Using Bleeding Time Tests: Anatomical Sites and Species Comparison


When studied using human induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CMs), ciluprevir increased both Ca2+ transient and impedance beat duration, and slowed the beating rate concentration-dependently starting at 11μM. It had no effect on Ca2+ transient amplitude, but associated to major, transient (+21%) increases in impedance amplitude. Ciluprevir 7-day treatment assays showed mitochondrial respiration at 100 μM and high-content imaging assay revealed that it affected mitochondrial intensity and texture, cytoplasmic and nuclear features at IC50s (50-65.4 μM) in hiPSC-CMs. While ciluprevir tested negative using HepG2 glucose/galactose medium switch assay, it acutely induced proton leak and showed an uncoupler-like profile (60 and 100 μM) while had no effect on electron transport chain activity up to 100 μM. The increased glycolysis is likely related to the metabolic adaptation to mitochondrial inhibition. In conclusion, MPA approach supports cardiac and mitochondrial disruption caused by ciluprevir. Uncoupler-like property corroborates with mitochondrial swelling observed in rhesus monkey heart tissues. Furthermore, this retrospective case study demonstrated the unique value of combining acute and long-term in vitro MPA approach as a tool for early de-risking cardiac and mitochondrial safety liability of new therapeutic drug candidates and facilitating preclinical candidate selection.
organ toxicity data exist.

**3306 Prediction of Tyrosine Kinase Inhibitor Contractility Risk with Adult Human Primary Cardiomyocytes**


Tyrosine kinase inhibitors (TKIs) provide effective cancer treatments by blocking kinases involved in tumour growth and angiogenesis, but they are often associated with cardiotoxicity ranging from heart failure, left ventricular systolic dysfunction and hypertension to arrhythmias. In order to enable the development of a new generation of safer TKIs, it is therefore critical to establish novel strategies that can help rank the cardiotoxicity of molecules in early drug discovery. Here, we have employed adult human primary cardiomyocytes from donor hearts and have shown that these cells exhibit the expected physiological properties and can be used for in vitro safety studies. In the present work, we have employed adult human primary cardiomyocytes to assess the cardiotoxicity of 7 US FDA-approved TKIs and one experimental TKI. Using clinical reference data, we have investigated the ability of non-invasive measurement of cardiomyocyte contractility to predict the cardiac safety risk associated with each one of the drugs. Both negative and positive controls were selected, including 4 known cardiotoxic drugs (AZD7762, Imitinib, Sorafenib, Vandetanib) as well as 4 non-cardiotoxic (Afatinib, Dasatinib, Erlotinib, Gefitinib). Our data demonstrate that isolated adult human primary cardiomyocytes are differentially affected by safe and cardiotoxic TKIs. For example, while Afatinib, Dasatinib, Erlotinib and Gefitinib had no effects on contractility up to concentrations equivalent to 30-fold the Cmax at therapeutic dose, AZD7762, Imitinib, Sorafenib and Vandetanib inhibited contractility with IC50 values of 0.8μM, 44μM, 1.2μM and 4.6μM, respectively, which closely match the therapeutic plasma concentrations. Thus, adult human primary cardiomyocytes can provide a useful strategy for the early assessment of cardiac risk associated with anticancer TKIs. Furthermore, this study resulted in the unexpected finding that human cardiomyocyte contractility can be affected by toxic TKIs, irrespective of the effects on the vasculature and chronic cardiac remodelling.

**3307 Trial to Detect Significant Metric Parameters and to Find Novel Methodologies for Drug-Induced Seizure Liability Using Microelectrode Arrays Data Analysis and Primary Rodent Neurons**

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Drug-induced seizures are a key reason for clinical failure and drug withdrawal from the market. Despite the huge safety problem, detecting seizure liability preclinically remains challenging and there is not yet a reliable, non-clinical, in vitro model to predict seizure liability of drug candidates. To address this, the study was designed to test effects of known compounds with defined seizure liability profiles on neuronal activity on the MEA, with the goal to identify activity signatures that associate with specific liability profiles. If yes, those profiles could be used to screen unknown compounds for seizure liability. Primary cortical neurons were prepared from fetal Wistar rats, 18 days post-coitum, and then seeded on 48-well Classic MEA plates and 24-well MED-Q2430M plates for extracellular recording using Maestro and Maestro Pro or using ME604-Presto respectively. Twelve compounds (pentyletetrazole, picrotoxin, 4-aminoypyridine, linopryridine, amoxapine, pilocarpine, amoxapine, chloromazone, oxinoklon, phentolamine and acetaminophen) were added to the cells (n = 6) at 5 concentrations for each compound to see phenotypic changes on spontaneous electrical activity in neural networks, after 19 days of culture. We identified consistent profiles of neuronal activity changes induced by compounds with similar modes of action. The structure of synchronized burst firing showed characteristic changes in neuronal activity patterns that were consistent with the effects found on significant metric parameter analysis. A deep learning algorithm could also successfully classify drugs by mode of action and accurately predicted synchronized burst response patterns in all drug types. MEA technology has the potential to predict the seizure liability of drugs using Analysis of Metric Parameters, Synchronized Burst Firing Structure, and Deep Learning Methodology.

**3308 Evaluation of a High-Content Cytometry Methodology for Drug-Induced Seizure Liability Using Microelectrode Arrays Data Analysis and Primary Rodent Neurons**

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Predicting in vivo toxicity liability with in vitro screens during early drug discovery could improve selection of lead series/single candidate pharmaceuticals while reducing animal usage, cost and time of chemical synthesis scale up. Therefore, high content, high throughput (HTS) assays using cultured cells, multiple toxicity markers, and machine learning methods have been used to correlate in vitro results with human and/or animal toxicity. Merck and AsedaSciences collaborated to use an HCS acute cellular stress assay, employing automated flow cytometry, to evaluate 115 compounds from discontinued Merck programs where in vivo organ toxicity data exist from short-term rat studies used for candidate selection. HLC60 cells were plated in 384-well plates at 10,000/well and incubated with duplicate 10-step dilution series of each compound for 4hrs, 5μM to 100μM. Unlike the traditional method of determining EC50 values for each of the 12 phenotypic parameters, an automated computational algorithm produces a set of distance values between controls and test compound concentrations for all 12 parameters. A supervised learning classifier then scores each compound relative to a training set of ~300 annotated failed and on-market pharmaceuticals and environmental toxicants. Results of the in vitro screen were compared to the in vivo rat outcome, i.e. organ toxicity assessed by gene transcriptional markers and/ or histopathology. In the in vivo studies compounds that were well tolerated, lacked organ toxicity, and achieved rat plasma Cmax exposures of ~50μM or higher were considered negative. Relative to the in vivo studies, the in vitro cellular stress screen exhibited 24% sensitivity and a high specificity (94%, only 5 false positives), desirable for an early drug discovery screen. In addition, there was high replicability for in vitro phenotypic parameters and scores in independent runs. Factors expected to limit HLC60 sensitivity include metabolism, transporters and ion channels, organ systems, and species differences (human vs rat). Overall, the results of this in vitro assay are promising for identifying intrinsic chemical potential of parent compounds to disrupt highly conserved cellular organelles. Predictive power will increase with growth of the training set and deployment of a parallel test strategy.
There is a strong focus on the development of new in vitro toxicity assays that are predictive of adverse events linked to drug attrition. These assays are often validated using only those drugs that have reached the clinical or market and for which adverse event profiles are known. For example, new hepatotoxicity assays are often validated using drugs contained within the Liver Toxicity Knowledge Base (LTKB). Whilst these ‘global’ validation datasets are critical towards understanding the human relevance of new assays, one of their limitations is that they are largely compiled of structurally-dissimilar compounds that have large variations in ADME profiles and pharmacological potency. These datasets are not reflective of the situation in early drug discovery where new in vitro assays must also be able to differentiate the toxicological profiles of compounds that may have similar structural and pharmacological profiles. For example, sunitinib, an anti-cancer multi-targeted kinase inhibitor, is classified as most-DILI concern, yet is associated with multiple organ injuries and is active in many in vitro assays. In contrast, aspirin, a compound of less-DILI concern is relatively pharmacologically inactive. An assay that can differentiate the hepatotoxicity profiles of these two compounds is clearly beneficial, yet it is possible that the assay is differentiating non-specific pharmacological potency as opposed to actual mechanistic, hepatotoxic potential. Therefore, in addition to a global validation, we conducted local analyses based on target class, mode-of-action and chemotype-similarity for new in vitro assays: 1) a human 3D liver microtissue model, 2) a proximal tubule cell model and 3) a human hematopoietic stem cell derived myeloid model. The importance of a local validation is clear, both the liver and myeloid model could distinguish internal compounds within same pharmacology and chemotype. For example, a drug candidate associated with bone-marrow toxicity had an IC$_50$ value of 0.11 μM in the myeloid model, whereas a non-toxic analogue of just two carbon difference was safer at 58 μM. This extra validation step is essential to ensure that activity observed in new in vitro assays is a result of toxicologically-relevant mechanisms and not the result of mechanistically-irrelevant pharmacological potency or physicochemical properties. In addition, local validation provides the evidence that assays can be used confidently to de-prioritize risky compounds at the design stage, thereby enabling a Safety-By-Design strategy.

Antibacterials and Nucleoside reverse transcriptase inhibitors (NRTIs) are known for their potential to induce mitochondrial dysfunction through depletion of mitochondrial coded proteins. The depletion of mitochondrial coded proteins can be achieved through effects on mitochondrial transcription or translation so direct evaluation of the protein expression is necessary to evaluate effects on both of these mechanisms. Existing high throughput methods utilize qPCR or inCell ELISAs to measure these effects. qPCR fails to determine effects on translation. InCell ELISA methods directly measure protein expression of one mitochondrial and nuclear coded protein in the cell culture well but it does not have the ability to quantify cell count and therefore could be misleading if the test article causes significant cell cycle arrest or cytotoxicity. Here we show development of a high content imaging assay which uses immunofluorescence to directly and simultaneously measure expression of the mitochondrial coded protein, COX-1, and the nuclear coded mitochondrial protein, SDH-A, on a per cell basis. Because of this, we are able to identify compounds that inhibit mitobiogenesis even when the cell count is reduced. Cell count can also be reported with the use of Hoechst in the nuclear channel. We evaluate the effects of 8 NRTIs and 8 antibacterials in 8-point dose response curves in a 384 well plate for 5 days. We show that we correctly identify the antivirals ddC and 4’ Azidocytidine (R-1479) as well as the antibiotics chloramphenicol and linezolid at their expected concentrations. For the positive compounds, we show a reduction in the COX-1 expression while we observe no loss in expression of the nuclear coded SDH-A. We also show that by generating a ratio of these two mitochondrial proteins, we can also create a much tighter curve as compared to the COX-1 alone. In conclusion, this assay is quantitative and reproducible, with the added advantage that cell count is determined simultaneously and results can be directly verified by analysis of the cellular images.
Drug-related mitochondrial toxicities are common off-target effects resulting in myriad of organ toxicities. High energy-demanding organs and tissues such as the liver are notably susceptible to the toxicities. Accordingly, hazards of mitochondrial toxicity are associated with drug-induced liver injury (DILI) in humans. To detect compound-related mitochondrial effects during lead optimization through candidate selection, companies employ in vitro assays including glucose/galactose switching assay (GGA), mitochondrial membrane potential/swelling, mitochondrial DNA and oxygen consumption rate measurements. Cell-based respiration studies that measure changes in OCR (OCR assay) are becoming a common tool and have potential to be more sensitive to identify mitochondrial toxicities than GGA. However, a thorough qualification of OCR assays has not been done yet in the context of DILI to date. Here, we evaluated a set of approximately 170 drugs with/without DILI in HepG2 cells using the Seahorse Extracellular Flux Analyzer. Compounds were preincubated at 1, 10 and 25 µM for 2h, followed by a standard protocol with minor modifications. Forty-five out of 173 compounds caused significant reduction in basal and maximal respiration (BR, MR) as compared to vehicle controls. Interestingly prospective uncouplers prevented reduction of OCR at certain doses after oligomycin injection, suggesting identification of dose-dependent uncoupling effects. For risk classification, we defined compounds causing >50% reduction in BR and/or MR as High Risk, <50% reduction as Low Risk, but as Medium Risk in case of obvious dose-dependent reduction in OCR observed. Results were compared to clinical DILI classification. OCR assay showed increased sensitivity (37%) and specificity (84%) as compared to GGA (21% and 80%). For comparison of each assay to identify mitochondrial toxicants from those that are not associated with clinical DILI, we calculated the positive/negative likelihood ratio (PLR/NLR) based on the sensitivity and specificity estimates. Higher PLR/lower NLR values were observed in OCR assay (2.22/0.76) in relation to GGA (1.01/1.00), supporting enhanced predictive value of OCR assay to correctly identify clinical DILI. When addressing only high dose drug (e.g. plasma Cmax > 1 µM), the predictivity of these assays increased with PLR/NLR values in OCR assay and GGA of 4.67/0.67 and 3.50/0.81, respectively. Taken together, the cell-based respiration assay outperformed GGA assay and appears a promising tool for prediction of DILI.

**3313 Evaluating the Utility of Microbrains Using Microelectrode Array, Calcium Oscillation and Calcium Imaging Technologies for Compound-Induced Seizure Risk Assessment**


Drug-induced seizure is the leading liability underlying the central nervous system toxicity attrition in drug discovery and development. To improve emerging in vitro methods to support compound-induced seizure risk assessment, we evaluated the utilities of human induced pluripotent stem (iPS) cellderived neural models (2D and 3D microBrains) using microelectrode array (MEA), calcium oscillation, and calcium imaging technologies. First, we characterized the microBrains, which were found to be evenly comprised of cortical neurons (GABAergic and glutamatergic) and astrocytes. Functionally, the 2D microBrains showed spontaneous firing on the low-throughput MEA platform. The 3D microBrain showed spontaneous synchronized calcium transient oscillations on the high-throughput Fluorescent Imaging Plate Reader (FLIPR) platform. Secondly, we selected rodent nonclinical seizure models using seizurogenic reference compounds, including but not limited to kainate, 4-aminopyridine, pentylenetetrazole, and bicuculline, to evaluate related genomic/proteomic biomarkers including expression level changes in markers of neuronal (e.g. UCHL1) and astrocyte toxicities (e.g. GFAP). Lastly, we examined whether the changes in MEA, FLIPR and/or biomarkers could be used to systematically detect seizure risk of proprietary compounds. Taken together, the 2D and 3D microBrains and associated biomarker changes showed promise as tools to reveal translational mechanisms of compound-induced seizure risk.
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