SOT
61ST ANNUAL MEETING
& TOXEXPO • SAN DIEGO, CA
MARCH 27–31, 2022

IMPORTANT DATES AND DEADLINES

MAY 17, 2021: Scientific Session and Continuing Education Course Proposal Submission Deadline

AUGUST 2, 2021: Registration and Housing Open

OCTOBER 9, 2021: SOT Awards Nomination and Application Deadline

OCTOBER 15, 2021: Abstract Submission Deadline; Undergraduate Awards 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 60th Annual Meeting of the Society of Toxicology, held virtually March 12–26, 2021.

An alphabetical Author Index, cross-referencing the corresponding abstract number(s), begins on page 336.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 359.

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Scientific Session Types:

- Continuing Education Courses
- Education-Career Development Sessions
- Informational Sessions
- Platform Sessions
- Poster Sessions
- Roundtable Sessions
- Symposium Sessions
- Workshop Sessions

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Chemical biology is an emerging scientific discipline that utilizes synthetic chemical probes to functionally identify and measure reactive biological molecules. Researchers design and synthesize small molecule chemical probes to functionally target and covalently label enzymes, receptors, and nucleic acids based on catalytic activities or selective affinities. Using fluorescent or mass spectrometry-based readouts, chemical probe platforms facilitate rapid and quantitative screening of cells, tissues, and biological fluids from microbes, animal models, and humans. Compared with conventional transcriptomics and proteomics, chemical probes provide measurements of functional activity rather than total abundance of transcripts, proteins, or nucleic acids. As such, chemical probes have recently gained popularity among research toxicologists and drug developers as tools to measure enzymatic activity important in metabolism and identify novel molecular binding targets of toxicants and drugs. This course will highlight innovative methods using chemical probes in the field of toxicology. The first presenter will cover how chemical probes can measure enzyme activity and resulting consequences of enzyme variability, induction, and ontogeny and impacts on chemical metabolism. The next presenter will demonstrate how chemical probes can be used to identify novel targets of organophosphates beyond acetylcholinesterase inhibition. Finally, the last presenter will discuss how chemical probes can reveal chemically induced damage to DNA and resulting mutations.

In recent years, single cell genomic analyses have provided a foundational new understanding of development and disease. While these novel and exciting technologies are being adopted across many fields in biology, their usage in the toxicological sciences is not yet widespread. This Continuing Education course will highlight the applications and current best practices for single cell genomics analyses in toxicology. The lectures will describe experimental design and analytic considerations for single cell experiments, define best practices and an overview of analytic methods for single cell RNA-sequencing and single cell chromatin profiling with ATAC-seq, and identify the state-of-the-art computational methods for integrated single cell multi-omics analyses and new machine-learning techniques to best apply single cell technologies in toxicology studies. The content of the course will benefit researchers from industry, government, and academia who evaluate mechanisms of action and safety of experimental compounds, consumer products, and environmental exposures and want to learn more about emerging technologies in this rapidly evolving area.

Understanding disruption of thyroid signaling pathways and thyroid homeostasis following exposure to environmental, agricultural, and industrial chemicals is both an evolving and an increasingly important challenge in the global regulatory community. This session will focus on innovative new approach methodologies (NAMs), such as 3D microtissues, organ-on-a-chip, hepatic thyroid clearance models, and computational approaches, that are being developed for predictive and mechanistic thyroid toxicology testing approaches. There is currently a heavy reliance on traditional animal testing approaches to evaluate the potential for a chemical to induce adverse thyroid effects, which are time and resource intensive. In fact, several in vivo guidelines studies were needed to identify additional thyroid endpoints, such as thyroxine and thyroid-stimulating hormone measurements. There is an opportunity to harness new transformative approaches, such as in silico screening and organotypic in vitro models, to replace animal-intensive testing programs to identify thyroid disrupting toxicants and elucidate the mode of potential action. Endogenous NAMs, such as in silico models, both provide valuable information to aid in molecule design from a predictive safety standpoint and provide guidance for targeted toxicological testing strategies. With continual progress in screening assays for thyroid hormone disruption as demonstrated by recent publications and new releases of data, and with the EU’s ongoing NAM effort to functionally identify the EU binding domain of thyroid disrupting chemicals, future progress in developing such assays to identify points of chemical interaction with the thyroid pathway, this session will provide a timely update on the data and tools available for rapidly evaluating in vitro activity relevant to the thyroid adverse outcome pathway network. To this end, experts from industry, the United States government, and the European Commission will discuss the current state-of-the-science and how these approaches are being utilized for predictive and mechanistic studies as well as regulatory toxicology applications. Each speaker will discuss opportunities for NAMs to be integrated in chemical safety evaluation. After the presentations, a Q&A will engage attendees to enable deeper understanding of the current state-of-the-art in approaches for addressing chemical-induced thyroid-related bioactivities. The target audience would be those interested in understanding how these tools are being leveraged in real-world regulatory testing paradigms. They will also gain insight into the strengths, limitations, and future development opportunities of in vitro, in silico, and alternative models for predictive and mechanistic thyroid toxicity assessments.

Advancement of metal toxicology, from a historical perspective, relies on innovation in science and technology. Discovery of atomic absorption spectrophotometry in the 19th century made it possible to quantify metals in the environment and human body, representing a turning point in understanding metals’ effects on human health. Since then, a variety of animal models have been developed—ranging from drosophila, C. elegans, and zebrafish to rodents and nonhuman primates—to assist prediction and mechanistic interpretation of metal toxicity and metabolism. Recent advances in specific fluorescent metal-binding ligands have further allowed tracing of the subcellular trafficking of metals by live imaging in cells and tissues. For mechanistic investigation, the CRISPR technology permits impeccably gene editing, lending itself to an effective, precise, and affordable method for identification of modes of metal toxicity. Moreover, big data algorithms and artificial intelligence (AI) offer advantages not only by the machine learning for fast processing of existing data, but more importantly through learning, it maximizes the chances of successful choices for better prediction of metal’s health impact. Achievements notwithstanding, application of these technologies—especially AI in infeotoxicology and CRISPR in biotechnology, two leading technology breakthroughs—in basic metal toxicological research remains in its infancy. This basic course is designed to introduce essential concepts and new technologies in the metal toxicology research field. The first lecture will review the history of metal toxicology in the context of technology advancement, followed by identifying gaps in the field and the future direction of trace element research. The second lecture will introduce the principles in metal quantification, with a focus on using genetic- and protein-based biomarkers for assessment of metals in cells and tissues; the speaker will also discuss fluorescent reporters and high-tech imaging and spectroscopy in metal research. The third lecture will discuss the concepts, general approaches, and applications of CRISPR for precise mechanistic study of metal toxicity; the speaker will teach this revolutionary technology from his own experience on the ideal procedure for investigation of metal-induced neurotoxicities. The fourth lecture will focus on the essential framework and concepts of AI for choosing the most appropriate molecular markers to study modes of metal toxicity, neurotoxic risk, and therapeutic treatment. Finally, the last lecture will introduce the basic concept and general practice of AI in health research, followed by integrative examples of how to use AI to interpret chemical toxicities as well as the policy regulation. Each lecture captures the most up-to-date knowledge and development in the field and discusses the concepts and technologies with details specific to metals that have particular human environmental and occupational health relevance, such as lead (Pb), manganese (Mn), cadmium (Cd), arsenic (As), silver (Ag), and mercury (Hg). The course will benefit those who desire to learn basic knowledge on this subject, for mechanistic interpretation, novel concepts and machine-assisted prediction of metal or chemical toxicities, and technical approaches in utilizing widely available CRISPR and cellular imaging technologies that can be used to support research in metal toxicology. As the course introduces these techniques that are equally applicable to other fields, such as neurotoxicology, nephtoxicology, carcinogenesis, risk assessment, and occupational health research, researchers engaged in these wider aspects of toxicological sciences shall benefit by attending this basic course and learning the knowledge beyond metals.
Design, conduct, analysis, and interpretation of mixtures experiments are daunting challenges. Frequently, defined-mixture experiments are performed, without knowing whether the response of a mixture is predictable from the dose-responses curves of the component chemicals. Experimental toxicologists have found that guideline study designs, while extremely valuable for intended purposes, are often not useful for investigation of consistency or lack of consistency with various definitions and forms of additivity (e.g., dose/concentration addition, response addition). Not typically taught in toxicology courses, individuals seeking knowledge on experimental design for mixtures generally sort through sometimes bewildering literature, where sources seemingly or actually, contradict one another. There is a long history of poorly designed studies and follow-up studies and lack of concordance of clinical endpoints in laboratory animals necessitates an understanding of the development, maturation, and function of the female reproductive system. The development, maturation, and function of the female reproductive system is of paramount importance, but there are many added layers if the product has sustainable attributes. Sustainable products are those that address current-day challenges of depletion of natural resources, high energy consumption, and release of chemicals and waste into the environment. Furthermore, sustainable products also are those for which consumers hold high expectations of having more transparency about the ingredients, production methods, and fewer instances of risk assessment. A certain level of satisfaction and product performance. Global regulatory agencies, academicians, product developers, and manufacturers have been working toward developing such sustainable, innovative, safe, efficacious, and cost-effective solutions for consumers. With advances in substituting existing substances and processes with greener alternatives, there is a need for holistic methodologies that ensure that the substituted products and processes leave a smaller environmental footprint throughout their life cycle. Toxicologists must integrate all these considerations into their product safety risk assessments. The Organisation for Economic Co-operation and Development (OECD) Publication For Maison, Growth highlights how the chemical industry and chemical management serve as examples of a scientific discipline that influences innovation in green technologies. As the demand for sustainable products increases, there is a need to integrate the elements of green and sustainable chemistry, such as green engineering, with toxicology early in the product development process. The field of “green toxicology” expands on the principles of green chemistry to develop products that not only are safe for use but also result in reduced human exposure, waste, or environmental impact; address climate change; and are not resource intensive. The US EPA Toxics Release Inventory and Safer Choice Program and USDA Biobased certifications highlight the shift toward ingredient safety and transparency, as well as the incorporation of 21st-century toxicological principles and advances with green chemistry to develop sustainable alternatives. This shift emphasizes the need for toxicologists to provide guidance on the requirements in the development of sustainable alternatives. When substituting with greener alternatives, toxicologists need to understand how to meet sustainability-related certifications and claims.

This CE course will provide an overview of the role of the safety assessment toxicologist in bringing sustainable solutions to the market, with case studies from different sectors. The speakers will present (1) the key principles of green chemistry and how they interact with toxicology, offering opportunities for toxicologists to be engaged with the selection of more sustainable ingredients; (2) US EPA programs such as the Toxics Release Inventory and Safer Choice, the national analyses that demonstrate the use of databases and assessment tools by toxicologists to identify and prioritize specific chemicals that, if replaced, can reduce the impact on waste streams in various industries; (3) the importance of understanding consumer expectations and how regulatory toxicology, external certifications, and safety-related product claims converge to inform the safety assessment of a sustainable product, demonstrated with a laundry detergent case study; (4) strategies for the application of in silico, in vitro, and targeted in vivo tests within the stage gate development process to satisfy regional and pseudo-regulatory requirements from retailers to produce more sustainable personal care products; and (5) the toxicological assessment considerations in the design and manufacturing of pharmaceuticals. Attendees of this CE course will be equipped to apply the key principles of green toxicology, use different tools and approaches, and navigate certifications to build safer products for sustainable consumer products and pharmaceuticals. In addition, this CE course provides the opportunity for attendees to learn about a transdisciplinary field, capitalize on scientific advancements in safety assessment, and discover the robust role of toxicologists in innovating sustainable products and practicing product stewardship.

1006 CE06: Insider Secrets for Design and Analysis of Defined-Mixture Experiments

J. E. Simmons, US EPA/CPHEA, Research Triangle Park, NC.

Design, conduct, analysis, and interpretation of mixtures experiments are daunting challenges. Frequently, defined-mixture experiments are performed, without knowing whether the response of a mixture is predictable from the dose-responses curves of the component chemicals. Experimental toxicologists have found that guideline study designs, while extremely valuable for intended purposes, are often not useful for investigation of consistency or lack of consistency with various definitions and forms of additivity (e.g., dose/concentration addition, response addition). Not typically taught in toxicology courses, individuals seeking knowledge on experimental design for mixtures generally sort through sometimes bewildering literature, where sources seemingly or actually, contradict one another. There is a long history of poorly designed studies and follow-up studies and lack of concordance of clinical endpoints in laboratory animals necessitates an understanding of the development, maturation, and function of the female reproductive system.

The development, maturation, and function of the female reproductive system is a complex, dynamic process in humans and laboratory animals and is sensitive to perturbation following exposure to a range of environmental and pharmacological agents. As a result, preclinical studies involving therapeutic interventions are those that address current-day challenges of depletion of natural resources, high energy consumption, and release of chemicals and waste into the environment. Furthermore, sustainable products also are those for which consumers hold high expectations of having more transparency about the ingredients, production methods, and fewer instances of risk assessment. A certain level of satisfaction and product performance. Global regulatory agencies, academicians, product developers, and manufacturers have been working toward developing such sustainable, innovative, safe, efficacious, and cost-effective solutions for consumers. With advances in substituting existing substances and processes with greener alternatives, there is a need for holistic methodologies that ensure that the substituted products and processes leave a smaller environmental footprint throughout their life cycle. Toxicologists must integrate all these considerations into their product safety risk assessments. The Organisation for Economic Co-operation and Development (OECD) Publication For Maison, Growth highlights how the chemical industry and chemical management serve as examples of a scientific discipline that influences innovation in green technologies. As the demand for sustainable products increases, there is a need to integrate the elements of green and sustainable chemistry, such as green engineering, with toxicology early in the product development process. The field of “green toxicology” expands on the principles of green chemistry to develop products that not only are safe for use but also result in reduced human exposure, waste, or environmental impact; address climate change; and are not resource intensive. The US EPA Toxics Release Inventory and Safer Choice, the national analyses that demonstrate the use of databases and assessment tools by toxicologists to identify and prioritize specific chemicals that, if replaced, can reduce the impact on waste streams in various industries; (3) the importance of understanding consumer expectations and how regulatory toxicology, external certifications, and safety-related product claims converge to inform the safety assessment of a sustainable product, demonstrated with a laundry detergent case study; (4) strategies for the application of in silico, in vitro, and targeted in vivo tests within the stage gate development process to satisfy regional and pseudo-regulatory requirements from retailers to produce more sustainable personal care products; and (5) the toxicological assessment considerations in the design and manufacturing of pharmaceuticals. Attendees of this CE course will be equipped to apply the key principles of green toxicology, use different tools and approaches, and navigate certifications to build safer products for sustainable consumer products and pharmaceuticals. In addition, this CE course provides the opportunity for attendees to learn about a transdisciplinary field, capitalize on scientific advancements in safety assessment, and discover the robust role of toxicologists in innovating sustainable products and practicing product stewardship.

1007 CE07: Development, Toxicology, and Pathology of the Female Reproductive Tract: Interpretation of Findings from the Pathologist and Regulatory Perspectives

A. Watson, Integrated Laboratory Systems Inc., Research Triangle Park, NC.

The development, maturation, and function of the female reproductive system is a complex, dynamic process in humans and laboratory animals and is sensitive to perturbation following exposure to a range of environmental and pharmacological agents. As a result, preclinical studies involving therapeutic interventions intended for use in the female population or agents with potential widespread human exposure often require toxicologic and histopathologic assessments of female reproductive endpoints to demonstrate safety. Evaluation of these endpoints in laboratory animals necessitates an understanding of considerations that include developmental timing, concordance of clinical and histopathological correlates, species differences, and the translational relevance of animal findings to the broader human population. The objective for this advanced CE course is to provide attendees with an overview of the development and maturation of the female reproductive system, study design considerations, and pathology and regulatory perspectives to facilitate interpretation of data for endpoints in vivo and animal studies. Speakers from academia, industry, and government (research and regulatory) with expertise in the fields of female reproductive and developmental toxicology will provide attendees with (1) a concise review of the development of the female reproductive tract, highlighting species differences and known targets; (2) current toxicologic and histopathologic methods to assess effects on female reproductive function and cyclicity; (3) distinct mechanisms of toxicity in adult female rats, including the onset of sexual maturity, cycling, and reproductive senescence; and (4) a regulatory perspective that will cover recent draft guidance from the US FDA and other regulatory bodies and in-vitro and in-vivo examples to illustrate specific issues risk-managed when reviewing preclinical toxicity packages for small molecules and biologics.

Note: this course will complement the CE course “The Male Reproductive Tract: Development, Toxicology, and Pathology,” presented as part of the scientific program during the 2020 SOT Virtual Meeting. The course “The Male Reproductive Tract: Development, Toxicology, and Pathology” is available as part of the SOT Ced-Tox, the Society’s online continuing education course program.
Drug failures in clinical trials are mainly due to the poor translational relevance and clinical predictive power of existing preclinical models, which include human cell-based in vitro and animal models. Microphysiological systems (MPS) (or organs-on-chips (OOC)) bring together advances in stem cell/organoid biology, biomaterials, tissue engineering, and biosensors to generate healthy and diseased models, where these human organ biomimetics more closely model the organ’s physiology. There is a clear need to enhance predictability of toxicities that may be encountered in human subjects. Human MPS models may assist to better identify early potential toxicity and elucidate the mechanism of toxicity once identified. The goal of the course will be to outline general principles and considerations of the appropriate use of OOC/MPS models in drug development for safety evaluation and highlight advantages/limitations in the current models. The first talk will give an overview and history of OOC/MPS. The tissue chip developer will discuss how to leverage MPS technology for generating toxicity assays and will give several examples of systems that have been used to evaluate toxicological events. The second presentation will focus on the characterization and validation of linked organ chip systems that could be utilized for PK/PD modeling and for a predictive way to model human drug toxicity. The third presentation will give insights and recommendations from a pharma perspective when implementing 3D/MPS for early toxicology testing and for later-stage toxicology investigations in a drug discovery setting. The final presentation will be given from a regulatory perspective that will inform the audience about performance criteria, standardizing the evaluation of MPS, and the importance of utilizing human cellular material and will present cardiac and liver MPS case studies. This course should be of broad interest to laboratories considering using 3D/OOC/MPS platforms as a mechanistic approach to predicting and understanding human organ system toxicities.

New chemical modalities (such as RNA-based or oligonucleotide gene therapies) represent a paradigm shift in drug discovery and toxicology. While these molecules were initially developed as therapeutics more than 30 years ago, novel sequences, chemistries, and delivery mechanisms have introduced unknown safety risks that require toxicologists to expand beyond the traditional small molecule chemistry space and think more broadly when assessing potential hazards and how toxicological effects will impact meaningful therapies for patients. This Continuing Education course will serve as a roadmap for how to approach evaluating safety concerns for novel oligonucleotides starting in early drug discovery phases through regulatory development, and will detail approaches to design oligonucleotide-based gene therapies with safety in mind. The course will begin with an overview that explores the advantages of oligonucleotide platforms over the last three decades and outlines the obstacles faced by toxicologists to evaluate safety for novel oligonucleotide sequences. Our first speaker will delve into chemical and structural sequence alterations associated with toxicity as well as share a case study that highlights the importance of sequence selection for optimizing tolerability. The next speaker will explore several studies that emphasize the importance of in vitro assays for predicting oligonucleotide-dependent toxicity and the utility of 3D microphysiological systems for de-risking oligonucleotide platforms. The final speaker will focus on available preclinical in vivo models for oligonucleotide toxicity studies and concerns regarding cross-species differences in response. The fourth speaker will discuss the preclinical and clinical oligonucleotide therapy landscape and findings from a meta-analysis study detailing the main adverse events driving attrition of oligonucleotide candidates in the clinic. The final speaker will conclude the course with discussion of regulatory approaches for novel oligonucleotide gene therapies and the advantage of pre-IND discussions to ensure successful development of novel compounds. Navigating a new chemical modality space can be challenging, especially when no defined regulatory pathway exists; therefore, this course offers a guide for the development of novel RNA-based therapeutic platforms from chemical toxicology through drug development. As experts in their field, the speakers offer key insights into drug discovery and toxicological parameters that are essential for successful development of oligonucleotide therapy platforms and will aid in advancing our understanding of unforeseen drug-induced toxicological endpoints for improved human health and safety.

Traditional chemical assessments are time-intensive, manual efforts requiring large amounts of human and experimental data. There are times, though, when a quick assessment of health impacts for a chemical is needed. Literature-based chemical assessments paired with computational and open-access (free) software applications (tools) can provide a quick, evidence-based solution. Human expertise supported by these tools can allow you to go from zero information to a preliminary hazard level without setting foot in the lab. Many freely available tools and workflows exist to support this process without adding on the cost for new software. This course will provide an overview of the types of chemical health safety assessments and their information requirements, setting the stage for how tools can support rapid chemical evaluations. It will close with an example of how structured data extraction is deposited into US Environmental Protection Agency (US EPA) dashboards. The format of the course follows the risk assessment process, as follows:

- **Identifying chemical information using literature search:** No little information on a chemical? Learn how to conduct a literature search for chemical data. Focus will be on search strategy, identifying studies with information of interest, and results prioritization.
- **Expanding the dataset using chemical similarity:** Are the data for the chemical of interest limited, but rich for a structurally similar neighbor? Learn how to expand your chemical search for data-poor chemicals using structural similarity or apply new approach methods in chemical assessment while considering other key data.
- **Using in vitro evidence for toxicokinetic modeling:** Is the evidence so limited (i.e., no information available at all) that a purely in vivo/in silico approach should be considered? Learn how to use in vivo to in vivo extrapolation to convert in vivo measurements into more reliable values of exposure scenarios. This will help you go from internal concentrations (or close estimates) to external/applied exposure concentrations/doses.
- **Calculation of point of departure:** Use the concentration response information from various data sources, including in vitro, in vivo, and in silico, to develop the point of departure (POD). You’ll need the POD to develop toxicity reference values that you can use to estimate safe exposure levels.

Course materials will include listing of additional resources, a walk-through of highlighted tools discussed, and example datasets. At the end of this course, participants will be able to:

- Explore and prioritize data needed for rapid chemical assessment
- Expand searches for data-poor chemicals using chemical analog toxicity information
- Extrapolate in vitro bioactivity to in vivo exposure scenarios
- Apply approaches for calculating POD

Over the past several decades, there has been a disturbing trend of declining efficiency in drug research and development. This trend has led to unsustainable cost growth for pharmaceutical research and highlights a significant risk for the development of new drugs. One of the most compelling explanations is that the conventional “brute force” methods of drug discovery are reaching a point of diminishing returns. Animal tests are too slow and expensive to keep pace with increasing demands for innovation and often fail to predict human responses because traditional animal models frequently do not accurately mimic human physiology. Organ-on-a-chip systems have the potential to address these concerns and meet the growing need for rapid, affordable, and replicable preclinical models. They offer the benefits of using human cells to recreate functions of living human organs, thus bridging the gap between extensively studied animal models and human clinical trials. As with any model, some level of confidence in the results provided is necessary for the successful implementation of organ-on-a-chip models, and widespread agreement in the field on approaches for the validation of organs-on-a-chip will be essential. This course will present considerations for the validation of organ-on-a-chip models for toxicity assessment from the perspectives of regulatory, industry, and academic stakeholders, with lung-on-a-chip as an example. The course Co-Chairs will begin the session with a brief introduction of the topic and the speakers. The first two speakers will be representatives of US government agencies. The first speaker, a representative from the US FDA, will discuss regulatory compliance and application requirements that significantly impact the use of organs-on-a-chip technologies for drug discovery and development. She also will describe current thinking on use of nonanimal alternatives in efficacy and toxicity testing. The second speaker, representing NIEMS and ICCVM, will focus on the challenges and lessons
learned from past and current validation efforts. The next three speakers, including representatives from academia, government, and industry, will present the perspectives of laboratories that conduct organ-on-a-chip research, development, and validation efforts. The third speaker will present the development and validation of a multi-organized “body-on-a-chip” platform for testing drug toxicity and developing countermeasures for toxic agents. Next, the fourth speaker will present the applications for and validation efforts with a multi-bioreactor platform that recapitulates bronchiolar and alveolar aspects of the human lung. Finally, the last talk will be a collaborative presentation describing the design and validation of a breathing lung-on-a-chip that integrates reliable and reproducible application of test aerosols at the air-interstitial interface. This course includes a diverse group of experts that will translate well to the target audience of scientists and practicing toxicologists. Attendees from academic institutions, government, and industry alike will be well represented and have sincere interest in the overall discussion. Attendees will leave the session with a greater understanding of the regulatory considerations, lessons learned, and potential next steps for the validation of organ-on-a-chip systems for toxicity testing.

**1012 CE12: Risk Assessment, DART, and Endocrine Disruption: A World View**

B. Hannas, Corteva Agriscience, Newark, DE.

Protection of humans from excessive exposures to chemicals and pharmaceuticals associated with toxicity can be managed through risk assessment. Developmental and Reproductive Toxicity/Endocrine Disruption (DART/ED) hazard identification (ID) is a critical component of the risk assessment process. DART/ED hazard ID also is used independent of exposure assessment to identify compounds with DART or ED properties and, in some cases, limit or prevent sales in certain geographies. Although risk assessment or hazard ID applications can differ across sectors and geographies, scientists often collaborate on best practices for methods and interpreting endpoints within DART and endocrine-specific toxicity studies. This course will therefore provide a view of the regulatory landscape for DART/ED assessments, focusing on specific cases and examples of applying DART/ED data to the end goal of protection of human health through risk assessment. The first talk will focus on the application of DART data for regulatory decision-making in the pharmaceutical sector. The second talk will then cover specific pharmaceutical case studies with DART data from nonclinical studies and the determination of human risk. The third talk will give an overview of endocrine disruption and how DART data apply to ED-specific requirements for chemicals across geographies, with examples of regulatory decisions based on existing datasets. The fourth talk will provide an overview of the US perspective on application of DART and ED data to the risk assessment process for chemicals, with a specific case study focused on thyroid assessments. Finally, the fifth talk will introduce alternative approaches for DART/ED assessments and the vision for application of alternative approaches to regulatory decision-making. This will be a crash course on the current regulatory approach to use of DART/ED data, with a view to the future, considering alternatives to animal testing approaches. As such, this course will offer broad appeal to audiences members of different backgrounds and may be of interest to trainees interested in a career in regulatory toxicology.

**1013 CE13: Timing Is Everything: Role of Aging in Immune Responses and Toxicological Implications**

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

Two major features in the process of aging of the human immune system are immunosenescence and inflammaging. Immunosenescence refers to the gradual deterioration of the immune system by natural age advancement and is one of the potential reasons for the increase in the incidence of infections. The term “inflammaging” was coined to combine the processes of inflammation and aging, since chronic, low-grade, systemic inflammation is associated with aging, contributing significantly to age-related diseases and mortality risk in the elderly. With age, the immune system undergoes adaptations and modifications, with important consequences for both communicable and noncommunicable diseases, for which the contribution of chemical exposure is not fully understood. This Continuing Education course aims to cover mechanisms of inflammaging and immunosenescence, their consequences, and implications in terms of response to vaccination, drugs, and immunotoxic compounds, which is timely and relevant in the era of COVID-19. The first speaker will introduce the audience to the current understanding of the biology underlying immunosenescence and inflammaging, and their contribution to age-related diseases. The second speaker will cover the problems associated with an effective vaccination and discuss how the understanding of immunosenescence will help in the design of more effective vaccines for the elderly. The third speaker will discuss the merits of animal models and their usefulness in the study of immunosenescence and drug-induced liability in a growing older population. Finally, the last speaker will cover the role of age in chemical-induced immunotoxicty and how the understanding of the mechanisms of action underlying chemical toxicity is central to define an increased risk—or not—in the elderly. Overall, this course aims to contribute to the understanding of physiological aging in the response to vaccines, drugs, and chemicals, which is considered of fundamental importance in light of an increasingly older population.

**1014 CE14: Understanding Tox21/ToxCast High-Throughput Screening Data and Applications to Modeling**

R. Huang, NIH/NCATS, Rockville, MD.

There is a large number of chemicals in the environment that lack adequate toxicological characterization necessary for the assessment of their exposure risk and subsequent regulatory decision-making. In order to generate toxicity profiles effectively on large sets of compounds, the US Tox21 and US EPA ToxCast programs have developed in vitro assays to test thousands of environmental compounds in a high-throughput screening (HTS) format. To date, more than 100 million data points have been generated from these screens and made publicly available. These datasets can aid in the identification of previously uncharacterized toxicants as well as the development of computational toxicity prediction models. However, there are technical aspects and caveats associated with these HTS assays that are not well understood by the end users, creating a gap between data generation and data interpretation. To bridge this gap, this Continuing Education course will provide an explanation and guidance on the understanding of Tox21/ToxCast HTS data to be applied more efficiently to toxicological modeling. The course will start with a presentation that describes various HTS assays used in the Tox21/ToxCast screening programs, followed by presentations describing different data processing methods and activity definitions dealing with biological and technological artifacts, a presentation comparing these data analysis methods, and finally a presentation on example applications to computational modeling. Live demos of the databases containing the results from different analysis pipelines will be included in some presentations. The content of this course will benefit researchers in the toxicology field, especially computational scientists who wish to develop models using the screening data and learn more about the assay technologies and data analysis methodologies.

**1015 Environmental Influences on Placental Origins of Development**

L. Aleksunes, Rutgers, The State University of New Jersey, Piscataway, NJ.

The placenta is one of the least understood human organs and is important, not only for the health of a woman and her fetus during pregnancy, but also for the lifelong health of both. The placenta connects the developing embryo/fetus to the uterine wall and functions as a barrier to mediate nutrient uptake, waste elimination, and gas exchange via the mother’s blood supply. It helps fights against internal infection, produces hormones to support pregnancy and metabolic activity, and transports environmental chemicals both within the placenta and to the fetus. Yet, our understanding of placental biology, endocrinology, and toxicology is very limited. It is becoming clear that the placenta is more than a conduit between the mother and developing fetus. It is a physiologically active tissue, which has the potential to impact the health of the offspring and the mother. The structure, including size, shape and orientation, and function of the placenta not only affect the health of the mother, as seen in the development of insulin resistance, preeclampsia, gestational hypertension, and eclampsia, but also affect the fetus, causing premature birth, intrauterine growth restriction, and functional changes in the fetus including altered male reproductive development and neurodevelopment. The goal of this session will be to discuss how toxicant exposures affect placental structure and function and how these changes impact the health of the offspring and the mother and offspring. This session will highlight placental research projects that use a combination of animal/cell models and noninvasive human placenta tissues or biomarkers from existing human studies to comparatively investigate placental exposures, and to gain a better mechanistic understanding of the effects of environmental exposures on early-stage placental health and the subsequent effects on the health of offspring.
The human placenta is a sexually dimorphic endocrine organ capable of metabolizing and synthesizing steroid hormones, which are necessary for trophoblast development, embryonic implantation, maintenance of pregnancy, and fetal growth, development, and well-being. Placental cells contain transporters and enzymes responsible for protecting the fetus from toxins/toxicants, however, we understand very little about their mechanisms of passage or the effects of exposures on placental and fetal development. These processes are partially derived from plastic. The purpose of this study is to quantify translocation through the placental barrier, identify anatomical particle deposition, characterize maternal and fetal health, and assess maternal hormone concentrations 24-hours after exposure. Whole animal studies provide visualization of significant polystyrene nanoparticle translocation in the placenta, fetoplacental plexus, and fetal viscerum in comparison to saline controls. In isolated placentas, we measured polystyrene translocation from the maternal-to-fetal compartment using our innovative placental perfusion system. Placental weights in the exposed groups were significantly increased, with no compensatory change in fetal weights. Dams who were administered polystyrene had significantly lower circulating concentrations of 17beta-estradiol and significantly higher hCGbeta levels compared to controls. There were no changes to progesterone or prolactin concentrations within 24 hours of exposure. Preliminary evidence shows the propensity for nanoplastic particles to translocate from the dam into the fetal compartment. Further particle exposure did not impact fetal growth but did impair placental efficiency. Lastly, exposure to nanoplastic particles during pregnancy may act to disrupt endocrine signaling.

Environmental exposures during pregnancy have implications pertaining to maternal and fetal health. One ubiquitous group of nanoparticles increasingly recognized due to their chemical stability and environmental accumulation are polystyrene. The purpose of this study is to quantify translocation through the placental barrier, identify anatomical particle deposition, characterize maternal and fetal health, and assess maternal hormone concentrations 24-hours after exposure. Whole animal studies provide visualization of significant polystyrene nanoparticle translocation in the placenta, fetoplacental plexus, and fetal viscerum in comparison to saline controls. In isolated placentas, we measured polystyrene translocation from the maternal-to-fetal compartment using our innovative placental perfusion system. Placental weights in the exposed group were significantly increased, with no compensatory change in fetal weights. Dams who were administered polystyrene had significantly lower circulating concentrations of 17beta-estradiol and significantly higher hCGbeta levels compared to controls. There were no changes to progesterone or prolactin concentrations within 24 hours of exposure. Preliminary evidence shows the propensity for nanoplastic particles to translocate from the dam into the fetal compartment. Further particle exposure did not impact fetal growth but did impair placental efficiency. Lastly, exposure to nanoplastic particles during pregnancy may act to disrupt endocrine signaling.

The placenta is a vulnerable target to environmental stressors and alterations in function underlie developmental disease and common pregnancy complications. While the developmental health risks remain undefined, legacy (polybrominated diphenyl ethers (PBDEs)) and emerging alternative (e.g., tetrabromobisphenol A, organophosphorous) flame retardants are identified at appreciable levels in the human placenta. Cytotrophoblasts (CTBs) play critical roles in human placentation and may be used to screen chemicals for their ability to cause placental toxicity in vitro. In our initial investigation, we exposed primary villous CTBs (isolated from 2nd trimester placentas) to BDE-47 and evaluated for potential effects on functional and toxicogenic levels. BDE-47 induced cytotoxicity (>10μM) and impaired CTB invasion/migration (5μM). On the transcriptomic level, BDE-47 significantly altered expression of 276 genes after a 24h exposure. Functional enrichment analyses of differentially expressed genes revealed significant changes in the cellular metabolism, morphogenesis, inflammation and differentiation to be disrupted by BDE-47. Validated targets included molecules relevant for environmental stress (e.g., IL6) and placentation development (e.g., MMP1). In parallel assessments, BDE-47 induced perturbations in global Cpg methylation, however, in general, differentially methylated Cpg regions did not correlate with changes in gene expression. In the next phase of our study, we used the CTB model to study the consequences of alternative FR exposures. Our initial results suggest multiple FRs may adversely impact the placenta by altering CTB function and disrupting common and divergent molecular pathways relevant to placental disease. This work was supported by the US EPA (R835082) and NIEHS (P01ES02841, RO1ES029386).
1021 Structure, Function, and Transporters in Brain Barriers: Implication in Metal Neurotoxicology

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

The integrity of the interfaces between the blood and brain extracellular fluids determines the fate of endo- and xenobiotics in the central nervous systems. This integrity is maintained by integral cellular structures formed by tight junctions between neighborhood cells and by effective transporters responsible for influx and efflux of materials. Damages to brain barriers’ structure, function, and transporters result in profound detrimental consequences in brain diseases and disorders. This presentation begins with a brief overview of structure and function of two brain barrier systems, i.e., the blood-brain barrier and blood-CSF barrier. The emphasis will be placed on the major categories of drug transport systems in brain barriers including restricted paracellular or transcellular passive diffusion, carrier-mediated transport, receptor-mediated endocytosis, adsorptive-mediated transcytosis, removal of drugs (efflux) from brain, and transnasal transport pathway. The presentation will discuss these transporters in the context of metal toxicities with speaker’s own experimental data specific to metals having particular human environmental health relevance, such as lead (Pb) and manganese (Mn).

1022 Clinical Imaging of Blood-Brain Barrier and Choroid Plexus in Brain Disorders

E. Canet-Soulas. Université de Lyon, Lyon, France. Sponsor: W. Zheng

Imaging blood-brain barrier (BBB) permeability is crucial in diagnosis and treatment of devastating neurological diseases such as stroke. This presentation will start with a discussion on the challenges of using neuroimaging technologies to evaluate BBB damage in the clinical context of emergency and patient follow-up. Magnetic Resonance Imaging (MRI) with the gadolinium-containing contrast agent is up-to-now the only recognized clinical method; it depicts the local barrier damage by qualitatively determining the contrast agent leakage outside the cerebral blood vessel. More advanced technologies such as dynamic contrast-enhanced (DCE) imaging have enabled the quantitative assessment of the permeability across the barrier. The speaker will provide both clinical and pre-clinical examples to demonstrate the importance of these measurements and their applications in evaluating the hemorrhagic risk in ischemic stroke. Since the imaging BBB permeability has gradually become essential to the personalized care in different brain diseases and to the follow-up evaluation of the treatment efficiency, the recent innovative methods and new contrast agents as well as their potential clinical applications will be specifically addressed. Finally, based on the recent data from the speaker’s lab using iron oxide or gadolinium nanoparticles, the possibility to explore the different blood-brain interfaces including the choroid plexus will be illustrated in both chronic and acute vascular situations.

1023 Developmental Specificity of Brain Barriers in the Context of Neurotoxicology and CNS Inflammation

J. Ghersi-Egea. INSERM U1028, Lyon, France. Sponsor: W. Zheng

Perinatal injuries from infectious, hypoxic or toxic origins can induce neurotoxicological damages ranging from dramatic abnormal brain development to neurodevelopmental disorders revealed later in life. The developing brain is not without protection, as blood-brain interfaces are efficient early in the fetal life to control brain fluid homeostasis and, to some extent, prevent toxic molecules from reaching the brain. Blood-brain interfaces, however, display developmental stage-specific properties that generate unique responses to inflammatory and toxic challenges, the subject that only recently starts to be understood. This presentation will present the evidence that the choroid plexus-CSF system plays a key, gender-independent role in protecting the brain fluid environment from harmful, pro-oxidant compounds during perinatal development when the astrocytes do not yet fulfill efficient neuronal protection, and when the detoxifying capacity of the liver is still immature. Evidences that lead and manganese accumulate in the choroid plexuses of the developing brain will be provided, and the impact of in-metals on selected choroidal barrier properties will be described. The speaker will review the postnatal windows of the susceptibility of the blood-brain interfaces that favors the propagation of systemic inflammation into the CNS.

Finally, strategies to enhance or preserve the integrity and antioxidant properties of brain barriers in order to improve the neurologic outcome of selected perinatal injuries will be discussed.

1024 Early Injury on Cerebral Vasculature: A Mechanism of TCDD-Induced Neurotoxicity in Zebrafish Models

J. Plavicki. Brown University, Providence, RI.

Brain health demands a continuous supply of oxygen and nutrients, while at the same time requires protection against blood-borne pathogens and toxicants. The blood brain barrier (BBB) is a combination of physical and chemical barriers that regulate the entry of blood-borne substances into the brain. The aryl hydrocarbon receptor (AhR) is necessary for mammalian vascular development; exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin), a potent AhR agonist and a global environmental contaminant, is known to induce vascular expression of cytochrome P450 1A1 (cyp1a), a widely accepted biomarker of AhR activation. This presentation introduces zebrafish as a new animal model to study the BBB developmental integrity and to test whether a global AhR activation by TCDD disrupts neurovascular development. Observations from this lab have showed that exposure of zebrafish to TCDD resulted in cerebral hemorrhaging, neurovascular malformations, loss of pericytes, and abnormal brain development. Using transgenic zebrafish and rt-qPCR, this group has also observed that the expression levels of glucose transporter 1 (glut1), a marker of differentiated brain endothelial cells with acquired barrier properties, as well as breast cancer resistance protein (bcrp), an efflux transporter expressed in brain endothelial cells, were significantly reduced following TCDD exposure, while p-glycoprotein (ppg) expression was unchanged. Further in this line of investigation, several transgenic fish lines have been generated to determine if endothelial-specific or neural-specific activation of AhR by TCDD is sufficient to recapitulate the observed phenotypes. The speaker will use her TCDD study as an example to highlight the advantages of using zebrafish models to visualize BBB development and function in real time for better understanding of the developmental genetics underlying barrier genesis, in order to develop the strategies for treatment of diseases or effective delivery of drugs to the brain by manipulating barrier properties, and to provide the insight into how toxicant exposures contribute to BBB dysfunction.

1025 Blood-Brain Barrier Damage in Traumatic Brain Injury and Metallic Nanoparticle-Induced Neurotoxicity


Traumatic brain injury (TBI) is one of the major causes of disability in the United States. It occurs when external mechanical forces induce brain damage that causes brain deformation. The initial trauma leads to neuronal, glial and endothelial cell death, an initial loss in barrier function and BBB permeability. The BBB is a physiological barrier consisting primarily of brain endothelial cells, which modulate the traffic of substances from blood to brain and vice versa. In vivo models of TBI using rodents have been developed to study the effects of TBI and to test potential therapeutic agents; however, it is important to move adequate in vitro models to aid in the early pre-clinical studies of TBI. This presentation provides an overview on the current effort to characterize the effects of mechanical stretch in rat brain microvascular endothelial cells, as an in vitro model of TBI to the BBB. In addition, effects of metallic nanoparticles (NPs) such as silver, gold, copper and iron on the BBB integrity and morphology will be presented. The speaker will use the recent data from this group to demonstrate the harmful effects of physical stretch and toxic insult by metallic nanoparticles on the cellular viability, expression of tight junction proteins and BBB permeability, and the correlations between in vitro and in vivo models. The data presented support the use of mechanical stretch as in vitro model of TBI to the BBB, since it replicates the damage observed in vivo after TBI and BBB as tool to study neurotoxicity of NPs.

1026 Industrial Applications of Artificial Intelligence in Toxicology

N. Greene, AstraZeneca, Waltham, MA.

Artificial intelligence (AI) is being touted as a solution to improve efficiencies in new product development. This is especially apparent in the chemical industries, where rising costs and greater regulations on environmental and consumer safety, as well as the desire or need to reduce the use of animals in testing, have led to major investments in AI. In the pharmaceutical industry,
computational methods in toxicology have received recent attention with the adoption of the ICH M7 Guideline, which specifies using (Q)SAR methods to predict bacterial mutagenicity of drug impurities, but computational methods in toxicology have had a much broader impact than just this narrow application. This session will look at a variety of applications of AI in chemical development using pharmaceutical development as an example. The methods and use cases demonstrate the diversity of computational uses that are dependent on real-world challenges toxicologists face. The first presenter will address how using large databases of in vivo data can be used to predict the outcomes of novel compounds using AI. The second presentation focuses on how artificial intelligence is aiding in the automated diagnosis of tissue damage resulting from chemical exposure. The third presentation will describe the use of graph convolutional networks to predict new indications for existing therapies, unwanted side effects of therapies, and address the concept of predicting the effects of combinations of drugs using real-world evidence. The final presentation will focus on predicting the pharmacokinetics of a drug molecule as well as its effects on a biological system using combinations of both structural and biological data including gene expression profiles.

1027 Development of In Silico Models from In Vivo Drug Toxicity Data and Their Successful Application for Regulatory Submission
A. Amberg, Sanofi, Frankfurt, Germany. Sponsor: C. Hasselgren

In vivo data, such as findings in histopathology and clinical chemistry examinations, are still the most relevant data that drive decisions in preclinical drug development and for regulatory submissions. In this context, we developed in silico models that were trained with findings from preclinical toxicity study reports for regulatory submissions. The sources of these in vivo data were previously used with in silico predictive toxicity models from 13 industry partners (1,947 drug candidates, 8,196 studies), approved drugs from PharmaPendium, as well as other publicly available data, e.g. the Leadscope database. These in silico models were individually developed for the main target organs, like liver, kidney and heart etc. The following compilation steps were applied to receive usable in silico model training datasets: Initially, the verbatim toxicity finding terms were harmonized using special ontologies. To receive model training sets with sufficient compound numbers and chemical space coverage, all primary histopathology terms were then combined to different clusters of similar toxicity mechanisms. For the most general clusters, terms were grouped into “tissue damage”, “inflammatory changes”, “structural alterations” or “accumulative lesions” clusters. Examples of more specific “tissue damage” cluster terms include “necrosis”, “steatosis”, “degeneration” etc. This resulted in a large number of training datasets for which different modeling approaches were applied, like structural alerts, fragment-based and molecular descriptor-based machine learning approaches. Validation studies showed that with this approach, in silico models could be developed to predict toxicity findings in main target organs for the use in preclinical drug development. Additionally, these in silico models have been successfully applied for regulatory purposes with the approval of the qualification of non-genotoxic Cymemazine impurities only based on results from in silico predictions with no additional in vivo toxicity data.

1028 Enhancing Toxicity Assessment of Histopathological Images Using Deep Learning
F. Hu, Genentech Inc., South San Francisco, CA. Sponsor: C. Hasselgren

Histopathological assessment of tissue changes in preclinical models of toxicity is an essential part of drug development. However, traditional histopathological assessment is based on qualitative evaluation, and findings are difficult to assess quantitatively. Therefore, grading and comparing toxicity among tissue sections is tedious in large toxicity studies. Moreover, traditional evaluation is premised on expert assessment, and inter-pathologist variation, a well documented phenomenon, makes it difficult to compare scoring across multiple studies performed by different pathologists over time. To enhance histopathological assessment and enable quantitative comparison, we separately tested several different deep learning models including segmentation, object detection, and multiple instance learning algorithms to quantify tissue characteristics as well as score for toxicity. Using segmentation algorithms, we quantified epidermal thickness (N=225) and retina thickness (N=325), and enabled rapid screening for degeneration in an in vitro epidemial model and mouse model respectively. Using object detection algorithms, we enumerated corpora lutea in rat ovary sections and also identified compound related toxicity consistent with that identified by study pathologists. The quantitative endpoints are not only reproducible but facilitate easy comparison across studies. With a multiple instance learning algorithm trained on both normal and abnormal rat livers (N=456), we achieved an 0.86 of area under the receiver operating characteristic curve to identify liver sections with toxicity findings in the unseen test set (N=153). With deep learning extracted quantitative endpoints and novel screening algorithms, our approaches contribute greater scientific insights into mechanisms of toxicity for faster and better drug development decision-making.

1029 Machine Learning for Prediction of Safe and Effective Drugs
M. Zitnik, Harvard University, Cambridge, MA. Sponsor: C. Hasselgren

The success of machine learning depends heavily on the choice of data features on which the methods are applied. For that reason, much of the actual efforts in developing algorithms go into engineering of features that support effective learning. This talk will describe our efforts to expand the scope and ease the applicability of machine learning for the prediction of safe and effective medicines. First, it will outline our methods for graph machine learning. The methods specify deep graph neural functions that map nodes in a graph to points in a compact vector space, termed embeddings. These graph neural methods are optimized to embed graphs such that performing algebraic operations in learned embedding spaces reflects the topology of input graphs. It will show how embeddings enable repurposing of drugs for rare diseases as well as new pathogens, for which no treatments are yet available. It will continue by describing our efforts in using graph neural networks to identify drug combinations that are safe in patients with considerably fewer unwanted side effects than today’s treatments. It will highlight how to develop methods that can predict and identify side-effects of individual drugs, novel drug combinations, and cocktails with two or more drugs. Lastly, it will describe our efforts in learning actionable representations that allow users of our models to ask what-if questions and receive predictions that are accurate and can be interpreted meaningfully.

1030 Toward Integrated Compound Safety Assessment, in Particular the Use of ‘Omics Data and Pharmacokinetics Information, in Toxicity and Safety Prediction
A. Bender, Cambridge University, Cambridge, United Kingdom.

Both pharmacokinetics (PK) of a compound and its local action need to be considered to understand its safety and toxicity profile in humans. The former describes exposure, while the latter can be more specific, such as activity on distinct proteins, but it also comprises unspecific activity, such as chemical reactivity, accumulation or changing pH in a local environment. With the increasing availability of PK data, ‘omics readsouts, and toxicity endpoint data we now have more data at hand, but their best use in many cases still needs to be established. In this contribution we will describe how in particular ‘omics’ data (such as transcriptomics data and cellular morphology screening data) can be used for the understanding and prediction of compound safety and outline what is needed for the further development of the field.

1031 A Future Framework for Application of In Vitro Metabolism and QIVIVE Models to Inform Risk Assessment
E. Haugabrooks, Physicians Committee for Responsible Medicine, Washington, DC.

Recent advancements of in vitro methodologies provide valuable insight into better ways to incorporate toxicokinetics and exposure in various decision-making contexts. One of the indisputable benefits of in vitro approaches is their capacity to increase chemical throughput compared with animal testing, allowing faster chemical screening and prioritization. However, with the advent of pressing global drivers to reduce and eliminate animal testing, understanding the utility of integrated in vitro approaches is critical. Often in chemical safety testing, when evaluating or extrapolating human kinetics from in vivo models, providing a comparison of effect levels from exposure scenarios has been challenging. Increasing evidence demonstrates we can move beyond the use of in vitro assays for compound prioritization and risk reduction and instead apply in silico approaches to quantify toxicokinetic and safety risk estimates by using physiologically based pharmacokinetic (PBPK) models and quantitative in vitro to in vivo extrapolation (QIVIVE). The advantage of using in vitro approaches now extends to dose-response relationships, benchmark dose modeling, and elucidating complex metabolic pathways. By leveraging an understanding of toxicokinetics and dose-response relationships, QIVIVE converts in vitro bioactivity through reverse dosimetry to estimate human equivalent administered dose. The objective of this session is to demonstrate,
through the use of integrated approaches, the application of in vitro metabolism and QIVIVE in various decision-making contexts. Key discussion points will include interpreting integrated data streams, building confidence in new approaches to inform quantitative risk assessment, applying weight of evidence to overcome assay uncertainty, and increasing uptake of new methodologies for regulatory application. Based on current findings, this session will also outline an initial framework to learn how in vitro technologies can be used to increase confidence in chemical safety and inform risk assessment. The session begins with a brief review of recent technological advances. Each speaker will then present a novel case study illustrating the benefits and complexities of adopting in vitro metabolism and QIVIVE models for chemical risk assessment.

**1032 Strategies to Overcome the “Human Metabolism” Bottleneck in Regulatory Risk Assessment of the 21st Century**

S. Coecke. European Commission Joint Research Centre, Ispra, Italy.

Sponsor: E. Haugabrooks

Although metabolism was originally considered to be the inactivation or detoxification of foreign compounds, today it is generally accepted that metabolism-mediated effects are an important issue in regulatory toxicity. Learning from historical examples of in vitro genotoxicity methods proposed for regulatory risk assessment, it is important for all in vitro methods dealing with toxic potency testing of compounds, to carefully consider human metabolism-mediated effects. The role of metabolism is particularly important when examining chemically induced target system effects, like thyroid system disruption. As such, kinetic processes and metabolism-mediated considerations need to be at the forefront of risk assessment strategies of the 21st century based on the integration of data generated from in vitro and in silico methods. To study metabolism pathways, assessing effects of chemicals on endogenous metabolism or identifying bio-activation, a multitude of reliable and relevant in vitro test systems can be used. With the help of the European Union Network of Laboratories for the Validation of Alternative Methods (EU-ENVAL) and a variety of international expert groups, several of these methods are being formally assessed and validated to be used for future decision-making. The presentation will give insight in the new generation of in vitro metabolism methods that identify chemical interference with the metabolism of thyroid hormones including investigations whether the effect is likely to be of human relevance. Using specific chemical, metabolism and biokinetic-dependent in vitro method indicators, the design of the most valuable and predictive integrated test strategies will be shown. The presented case study aims to stimulate discussion on the way forward to progress new risk assessment strategies for capturing systemic toxicity effects and will show that adequate harmonized characterization, description and integration of the next generation of in vitro and in silico methods and global coordination of efforts is critical in this process.

**1033 Toxicokinetic Models for QIVIVE and Considerations of Uncertainty**

X. Chang. Integrated Laboratory Systems Inc., Research Triangle Park, NC.

To effectively incorporate in vitro assay data into regulatory use, confidence must be established in the quantitative extrapolation of in vitro activity to relevant endpoints in animals or humans. Sources of variability and uncertainty in IVIVE approaches need to be considered in interpretation of IVIVE results. Potential sources include: the in vitro assay active concentration, inter-individual variability in animal or human physiology, and uncertainty associated with chemical-dependent toxicokinetic parameters (e.g., fraction unbound to protein and metabolic clearance, both measured and predicted). Open-source tools, such as HTTK-Pop have been developed to simulate human physiological variability and associated measurement uncertainty in toxicokinetic parameters. Using case studies and applying the HTTK-Pop tool, we assessed the impact of variability in IVIVE input parameters by comparing the range of possible estimated equivalent administered dose values to in vivo effect levels for in vitro assays and in vivo studies measuring the same biological pathways. We also explored a QSAR approach that incorporates uncertainty in the parameter predictions. This talk highlights how variability can impact confidence and quantification of IVIVE approaches.

**1034 Practical Applications of QIVIVE (Quantitative In Vitro to In Vivo Extrapolation) and Associated Interpretive Opportunities**

K. Magurany. NSF International, Ann Arbor, MI.

Integrating dose-responsive mechanistic evidence from in vitro assays that are aligned with adverse outcome pathways (AOPs) provides a framework for movement away from animal testing in the derivation of oral reference doses (RfD) for chemical contaminants that may be present in drinking water. Presently, practical application of these data present interpretive challenges for derivation of an RfD where apical endpoints from conventional animal studies are not well aligned with existing AOPs and uncertainties are not well characterized for the assays or the toxicokinetic model outcomes for quantitative application. This talk will highlight some of these challenges for the ToxCast endocrine model and the successful application of these data and the QIVIVE in a weight of evidence evaluation. Specifically, the process for evaluation of ToxCast in vitro data and the relevance of these data to characterize chemical hazard and to inform dose-response in the context of two case studies—benzophenone and p-t-butylphenol—will be presented.

**1035 The Impact of In Vitro Metabolic Competency on Chemical Screening and Its Relevance to In Vivo Toxicity**

S. Marty. Dow, Midland, MI.

Understanding metabolism plays a key role in the application of non-animal alternative methods (NAMs) to ensure that metabolite bioactivity has been adequately considered for chemical safety screening. In vitro metabolic assays, which can include subcellular fractions (such as microsomes), organ-on-a-chip, hepatocytes or targeted enzymes, can be used to examine metabolic rate and metabolic fate to generate intrinsic clearance for in vivo data; facilitate read across for safety evaluations of classes of chemicals; improve toxicokinetic modeling by refining intrinsic clearance estimates; and identify human-relevant metabolites, which can be screened for bioactivity using in silico or in vitro models. This presentation will briefly examine case studies showing the application of in vitro metabolism to facilitate the use of NAMs, including how intrinsic clearance can be used to refine in silico toxicokinetic models and improve in vitro-to-in vivo extrapolation of dosimetry. An example of the application of in vitro toxicokinetics for read across also will be shown. In vitro metabolism can be used iteratively to identify potentially reactive metabolites that can be predicted using QSAR models or to identify substances where metabolism is critical to accurately assess toxicity. Some gaps in metabolite consideration that can result in the under-prediction of test substance toxicity will be highlighted to illustrate the importance of metabolism to in vitro assay results. As we move into the future, coupling in vitro metabolism with in vitro toxicity assays will allow more thorough toxicity screening by accounting for metabolites rather than limiting in vitro screening to parent substances. Furthermore, in vitro metabolism data can improve QSAR and toxicokinetic models to support a more complete analysis using integrated approaches to testing and assessment (IATA).

**1036 Case Study Examples Establishing the Utility of QIVIVE and for Estimating Safe Human Exposures**

R. Clewell. 21st Century Tox Consulting, Durham, NC.

Ultimately, chemical risk assessment decisions are made based on an understanding of the relationship between potential exposure and the dose at which a chemical is likely to cause biologically effects, the Margin of Exposure (MoE). The last decade has been witness to a proliferation of computational and in vitro methods to estimate various routes of exposure, toxicokinetics and chemical dose-response that range from ultra-high throughput computational methods for rapid predictions and high throughput in vitro screening assays to highly complex 3-dimensional reconstructed tissues that allow testing in a highly biologically relevant system. As these methods are incorporated into decision-making, we must account for inherent uncertainty in these methods, aware that speed and efficiency are often gained at the expense of accuracy. Thus, implementation of NAMs into risk assessment will likely be achieved through a tiered approach that allows for prioritization and lower throughput, more biologically complex methods for quantitative risk assessments. At lower tiers, high throughput in vitro to in vivo extrapolation (HT-IVIVE) and HT bioactivity screening may be used for prioritization, while at higher tiers quantitative IVIVE and organotypic AOP-driven assays may be used to predict regions of safe human exposure. This presentation will illustrate this tiered approach through case study examples. The tiered risk assessment frame-
work will be presented. Case studies with estrogenic and anti-androgenic chemicals will be used to demonstrate translation of the framework to real-world scenarios with low and high throughput assessments using Q-IVIVE and HT-IVIVE and comparing resulting risk estimates to those derived from traditional animal methods.

1037 Chemical-Induced Mouse Lung Tumors: Mode of Action, Relevance, and Risk Assessment

Z. Yan, Corteva Agriscience, Indianapolis, IN.

Chemically induced lung tumors are commonly reported in mouse carcinogenicity bioassays, and often in the absence of parallel tumorigenicity in rats. Long-term bioassays need to be assessed for appropriateness of the dose, neither exceeding the Maximum Tolerated Dose (MTD) nor the Kinetically Based Maximum Dose (KMD). In addition, since mouse lung tumors are common (>1% incidence), the appropriate statistical significance is p < 0.01. Numerous differences exist for mouse lung tumors compared with humans, including anatomy, respiratory rate, metabolism, tumor histogenesis, and metastatic frequency. The recent demonstration of the critical role of mouse CYP2F2 metabolism in mouse lung carcinogenicity (e.g. styrene, fluensulfone) indicates that this tumor response is not qualitatively or quantitatively relevant to humans. For non-DNA reactive and nonmutagenic carcinogens, the mode of action involves direct mitogenicity (isoniazid, styrene, fluensulfone, permethrin) or cytotoxicity with regeneration (e.g. naphthalene). However, the possibility of mixed mitogenic and cytotoxic modes of action cannot always be excluded. Finally, human health risk assessment can be conducted based on these considerations/conclusions. This Workshop will comprise a series of presentations addressing (1) important considerations for assessing carcinogenic potential of chemicals due to increased mouse lung tumors; (2) discussion of inter-species differences in lung and respiratory tract anatomy and cell types, as well as metabolism between mice, rats, and humans, and thus a possible mouse lung tumor screening strategy; (3) utility of genetically modified mouse strains as mode-of-action research tools to understand key metabolic drivers of mouse lung toxicity and tumors; and (4) mode of action as a case example of a potential qualitative difference in mode of action between mice, rats, and humans, and (5) perspective from the US EPA; key considerations for regulatory decision-making. The Workshop presentations and panel discussion will catalyze audience dialog of the key mode-of-action consideration of “how much (data) is enough” and a potential decision tree to support science-justified risk evaluations of the human relevance of mouse lung tumors.

1039 Assessment of Mouse Lung Tumorigenesis: Importance of Evaluating Mode of Action and Relevance to Humans

S. Cohen. University of Nebraska Medical Center, Omaha, NE.

Mouse lung tumors are commonly induced by a variety of chemicals, but it remains questionable as to their relevance to human cancer risk. Although, there are similarities between human and mouse lung, there are several differences, and there are also several differences in the types and behavior of tumors that are produced. Modes of action that have been identified for mouse lung tumorigenesis include DNA reactivity and increased cell proliferation, which can be due to either cytotoxicity and regenerative proliferation or direct mitogenicity. Isoniazid (INH) is a typical example of a potent mouse lung tumorigen which acts through direct mitogenicity on club cells leading to increased incidences of lung adenomas. This anti-tuberculosis drug has been extensively investigated epidemiologically, and shows no evidence of an increased risk of lung cancer or other types of cancer. This and other examples in which epidemiologic studies have been performed strongly suggest that mouse lung tumors may not be relevant to human cancer risk. Utilizing these modes of action, short-term assays (less than two weeks) can be performed to screen for potential mouse lung tumorigens, as well as an evaluation of potential human relevance.

1040 Genetically Modified Mouse Models as a Means for Informing the Human Health Relevance of Mouse Lung Toxicants

X. Deng. University of Arizona, Tucson, AZ.

Genetically-modified mouse models and their application for investigating chemical-specific mode(s) of action and human relevance will be introduced and discussed in this presentation. The genetically-modified mouse models to be introduced include various CYP-null models, CYP-humanized models, as well as models with tissue-selective knockout of the P450 reductase gene. Applications of these mouse models for studying the metabolic mechanisms of lung toxicants and carcinogens will be presented, focusing on naphthalene and tobacco carcinogens. Mechanistic aspects relevant to risk assessment, including relative importance of target tissue and systemic metabolism, of different reactive metabolites of varying stability, and of different metabolic biomarkers will be discussed. These mechanistic considerations will be examined in context to informing human relevant risks of these agents.

1041 Styrene-Induced Lung Tumors: Lack of Quantitative or Possible Qualitative Relevance to Human Risk

J. Bus. Exponent, Midland, MI.

Recently completed styrene mode of action (MoA) studies have demonstrated that its mouse-specific lung tumorigenicity is primarily mediated by mouse lung-specific club cell CYP2F2 metabolism to an oxidized mitogenic metabolite(s). The data supporting this MoA will be contrasted to the dataset supporting the long-held alternative hypothesis that styrene mouse lung tumors are mediated by a genotoxic and regenerative cytotoxic mode of action associated with formation of 7,8-styrene oxide (SO) generated primarily by liver and possibly lung CYP2E1 metabolism common to both rodents and humans. The CYP2F2 MOA is supported by complete attenuation of short- and long-term styrene mouse lung toxicity in CYP2F2 knock-out (KO) and CYP2F1 humanized mice; importantly, SO lung toxicity in wild-type mice also was absent in CYP2F2 KO and CYP2F1 mice, indicating SO IS NOT the proximate lung toxic metabolite of styrene as previously hypothesized. Supplemental transcriptomic responses observed after short- and long-term styrene exposures revealed no evidence of a genotoxic MoA but clear evidence of mitogenicity and longer-term disruption of circadian clock genes, which have been identified as a driver of mouse lung terminal bronchiole lung tumors. Short-term transcriptomic responses also were completely attenuated in CYP2F2 KO and CYP2F1 humanized mice, indicating that lung metabolism by CYP2F2 is an initial key molecular initiating event in mouse specific lung tumorigenicity. The overall styrene MoA dataset indicates styrene mouse lung tumors are likely quantitatively and possibly qualitatively not relevant to human health risk. Importantly, the styrene-specific mouse lung tumor MoA likely is common to several other mouse-lung specific carcinogens such as coumarin, ethylbenzene, cumene, naphthalene and others.

1038 Introduction: Chemicals That Induce Lung Tumors in Mice but Not in Rats, and Biological Considerations for Evaluating Chemical Carcinogenic Potential

Z. Yan, Corteva Agriscience, Indianapolis, IN.

Lung tumors are observed in mouse bioassays by a variety of chemical and are often the basis for cancer risk assessment on these chemicals, including agrochemicals, industrial chemicals, food ingredients, cosmetics, consumer products and pharmaceuticals. Chemicals which were tested in the National Toxicology Program (NTP) and other organizations in both rats and mice but only produced lung tumors in mice through 2008 will be introduced. This presentation will then focus on a couple of key considerations important for assessing chemicals’ carcinogenic potential. First, it is essential to take into account the doses that are used do not exceed the maximum tolerated dose (MTD). As a logical extension, kinetically-derived maximum dose (KMD) approach identifies excessive biological stress by evidence of onset of non-linear TK associated with saturation of metabolic and clearance pathways. Consistent with MTD, toxicity observed only at high doses exceeding KMD, and that are well separated from real-world human exposure are not relevant to human hazards or risk assessment. Second, statistical considerations are critical. Mouse lung tumors are extremely common tumors in mouse strains in which the chemicals have been tested such as 8–32% combined adenoma/adenocarcinoma incidences in male B6C3F1 mice and 8–10% in female mice, thus application of a statistical analysis to minimize false-positive outcomes is essential. Haseman (1983) and US FDA suggested that a more appropriate value for statistical significance for common tumors (defined as those with a background incidence greater than 1%) be set at p < 0.01 for pairwise comparisons.
1042 The Perspective from US EPA: Key Considerations for Decision-Making


The US EPA Office of Pesticide Program (OPP) requires carcinogenicity testing in two rodent species for food use pesticides and when the use of the pesticide is likely to result in significant human exposure or a considerable portion of the human life span. OPP’s Cancer Assessment Review Committee (CARC) uses a weight of the evidence approach to evaluate the carcinogenic potential of pesticide active ingredients. Evidence considered by CARC includes adequacy of the doses tested, statistically and/or biologically significant non-neoplastic and neoplastic findings, metabolism data, genotoxicity data, structure activity relationships, and mode of action (MoA) data. Non-guideline mechanistic studies are frequently submitted to the agency to support a proposed MoA for a specific tumor type. These data are evaluated in the context of the 2005 EPA cancer guidelines and is consistent with the International Program for Chemical Safety (IPCS) 1998-99 MoA framework to determine links between postulated key events and the induction of tumors. This presentation will include examples of regulatory decisions for mouse lung tumors based on MOA data and data used to inform the adequacy of dosing. Examples will include the insecticide Fipronilam and the fumigant 1,3-dichloropropene (Telone).

1043 Establishing Quality, Safety, and Regulatory Principles for Probiotics: More Than Just a Gut Check

A. Roe. Procter & Gamble, Cincinnati, OH.

Probiotics are among the most widely used specialty supplements in the United States, according to a recent consumer survey conducted by the Council for Responsible Nutrition. A continued increase in probiotic usage is expected as companies develop probiotic strains for uses beyond gut health to such usage as sports nutrition and upper respiratory health. Like many natural products used in dietary supplements, probiotics are presumed to be safe. This presumption of safety is not completely unfounded: there is global consumption of common probiotics, and data from large cohort studies indicate a lack of serious adverse events. However, as probiotic manufacturers are increasingly seeking to use new strains, species, or even novel probiotics (human commensals, but not currently found in the food supply), justification for continued regulation becomes even more critical. This presentation will present the USP Expert Panel’s graphs for 7 probiotic strains, developed a general chapter for probiotic tests and the USP Expert Panel’s results in the United States. Because of the broad usage of the dietary supplement products among both adults and children and the absence of pre-market approval, there is a clear need for quality standards for dietary supplements. To that end, the United States Pharmacopeia (USP) has developed monographs for 7 probiotic strains, developed a general chapter for probiotic tests and convened a Probiotics Expert Panel. The Panel’s role is to provide input into the appropriate quality standards for probiotic dietary ingredients and dietary supplements. The Expert Panel is tackling issues related to probiotic identification, enumeration and safety. Two subteams have been formed: one subteam is working on DNA identification of strains; the second subteam is identifying the parameters and criteria to determine the safety of a probiotic. In addition, the Expert Panel published a paper “Improving End-User Trust in the Quality of Commercial Probiotic Products” covering third party certification programs, as well as identifying issues related to identification, genetic drift, limitations of quantification methods and challenges with single strain vs. multi-strain products. This presentation will cover the USP Expert Panel’s recommendations for quality and safety standards associated with probiotic dietary supplements.

1044 An Industry Perspective on Assessing Safety of “Biotics” as Dietary Supplements

A. Roe. Procter & Gamble, Cincinnati, OH.

Global sales of biotics (pre- and probiotics) were over 50 billion US dollars in 2018; and similar to other categories of dietary supplements projected sales will continue to increase in subsequent years. With increased interest in the use of biotics in dietary supplements it is important to establish a pragmatic approach for ensuring the safety of these ingredients. With this in mind, this talk will introduce the audience to a tiered approach when assessing the safety of probiotics and a decision tree approach utilized in evaluating the safety of probiotics. Briefly, tier assignment is based on an established history of safe human use at or below intended dosing in the target demographic, and review by an authoritative body, e.g. EFSA or FDA (GRAS dossier). Testing needs (in vitro/in vivo/clinical) are defined by tier. Data requirements follow international guidelines as outlined by FAO/WHO (2002) including antibiotic resistance profile, transmissible genetic elements, toxin production, and positive strain ID. Additionally, full genome sequence with assessment of protein coding and metabolic capabilities is recommended as the first step. For prebiotics and postbiotics, we have adapted a decision tree approach to assist in determining the information that is needed for the safety evaluation of a probiotic. Information required relies on a benchmarking to existing dietary intakes and is justified by exit points from the decision tree. It will always include specification of the product, details of the source, evidence from previous human exposure through food or other sources, extent of use and estimated intake. In addition to the prebiotics and probiotics contained within a formulation, other ingredients such as processing aids, cryoprotectants, impurities, preservatives etc are assessed for safety using traditional assessment methodology. This holistic approach to assessment enables us to ensure the safety of these products.

1045 Development of a Best Practices Guideline for the Review of Dietary Ingredients through the Self-GRAS Pathway


The Generally Recognized As Safe (GRAS) notification program is a voluntary mechanism through which FDA may be informed of a determination that the use of a substance (ingredient) in food is safe, following a thorough safety review. Whether done through a GRAS Notification, which is submitted to the FDA, or through an independent GRAS process (i.e., self-GRAS) the same scientific rigor must be applied to both the evaluation and review of the ingredient. In either case, information is required on ingredient identity, method of manufacture, physical or technical effects, dietary exposure and self-limiting levels of use. Further, studies performed in support of the safety of an ingredient must be published in a peer reviewed journal, thus providing transparency of key data utilized in support of marketing. One advantage of the self-GRAS process is that it allows companies to protect proprietary information – in the case of GRAS Notifications submitted to FDA, other companies can utilize information provided by the innovative company giving them an unfair competitive advantage. To date, the self-confirmed GRAS path has proven safe and, in many cases, FDA has been informed of self-GRAS affirmations (the Flavor and Extract Manufacturers Association voluntarily informs FDA of its GRAS determinations, including the name of the substance, its properties, and the basis of the determination). No instances of a self-affirmed GRAS substance being removed from the market due to an originally undetected potential safety concern have been identified. However, criticisms of the process have been leveled, including a lack of transparency and accusations of bias within expert panels convened to review the available data and decide on the safety of a substance/ingredient. Members of the Consumer Healthcare Products Association (CHPA) Dietary Supplement Committee have recently convened to develop a best practices document addressing criticisms associated with the self-GRAS process; details of which will be discussed in this presentation.
The Canadian oversight of probiotic products under the Natural Health Product Regulations demands comprehensive evaluation of the strains involved but remains dependent on well-known health claims. All probiotic products are expected to comply with stringent quality parameters and product specifications for each strain within a product. However, for scientifically well-known probiotic species, the Natural and Non-prescription Health Products Directorate (NNHPD) has pre-cleared certain general health claims at the species level and also streamlined safety and quality requirements at the strain level via a guidance document, the NNHPD Probiotics monograph. This NNHPD probiotics monograph exists to aid industry in complying fully with regulation in terms of safety, efficacy, and quality. It also allows industry to attain market authorization within a reasonable amount of time. More importantly, in conjunction with general guidance on safety and efficacy for Natural Health Products, it helps to provide a dividing line for the regulator between lesser known products needing more in-depth assessment and those that do not. This approach has resulted in a highly efficient and well-regarded regulatory framework for probiotics that is both industry and consumer friendly, while still allowing for in-depth safety assessments as required. The benefits of such a model and its limitations are discussed. In addition, regulation related to the emerging category of postbiotics as well as plans to address the nomenclature changes to Lactobacilli outside of regulation will be presented.

Human exposure to airborne substances occurs from a number of sources, including air pollution and the use of consumer products. As some of these exposures can have detrimental effects on health, exposure limits have been established, and/or registration is required for their use based on a thorough understanding of their acute and chronic toxicity. Traditionally, animal models have been used for assessing the respiratory toxicity of chemicals inhaled as aerosols, vapors, or airborne dust. However, the fundamental differences in the anatomy, physiology, and biology of animals and humans, along with ethical issues raised with animal testing, have greatly promoted the use of human-relevant in vitro methods. However, conducting in vitro exposures in a manner simulating human inhalation exposure is technically challenging due to the unique variables related to the properties of the test articles, exposure dosimetry, and deposition dynamics within the respiratory tract, all of which influence the outcomes of the studies. Although workshops have been held to establish consensus on in vitro methodologies for assessing inhaled substances, most discussions focused on cell-based lung models. Methods for controlled generation and delivery of test articles in forms and at doses relevant to human inhalation exposure, which are paramount to the resulting toxic effects of the test substances, are less discussed. Furthermore, the standardization of in vitro exposure methods that is required for their regulatory acceptance is also lacking. To stimulate discussions on this important aspect of in vitro inhalation toxicology, subject-matter experts will present their approaches for exposing in vitro human lung models to occupational chemi- cals, consumer products, and nanomaterials at the air-liquid interface (ALI). Specifically, Workshop speakers will present (1) an overview of the existing ALI exposure systems as well as the recommended practice for conducting analytical validation of these systems; (2) key considerations for selecting the appropriate in vitro test system, exposure regimen, and toxicity endpoints for toxicity assessment of occupational and consumer product exposures; (3) an integrated in vitro approach for safety evaluation of fragrance ingredients in consumer products; (4) a six-step methodology for selecting in vitro doses reflecting in vivo internal doses at the target organ burden for respiratory toxicity evaluation of nanomaterials; and (5) ongoing regulatory activities at the US EPA’s Office of Pesticide Programs to evaluate in vitro alternatives for inhalation risk assessment to support pesticide registrations. The audience will take home the basic experimental designs and practical considerations for testing a range of substances, the human relevancy of the approaches, and determination of exposure dosimetry. The roundtable discussion following these presentations will discuss elements that require standardization for regulatory acceptance and identify and prioritize common elements of the experimental approaches for conducting ALI exposures to airborne substances, regardless of their physiochemical properties, for further action. This Workshop will not only provide a comprehensive overview of methodologies for conducting in vitro inhalation exposures, but also identify key knowledge gaps in standardization of in vitro exposure methods for regulatory acceptance and develop a framework to ensure human relevancy of the in vitro approaches.

Inhaled toxicants have the potential to cause adverse portal of entry and systemic effects, therefore accurate prediction of these outcomes is a critical component of the evaluation of human risk and setting of safe exposure guidelines for these materials. While assessment of inhaled substances has been traditionally accomplished using in vivo rodent models, there is a need to develop relevant, non-animal alternatives for assessment of these materials; however, the complexity of the respiratory tract, its interaction with other tissue/organ systems and the unique features of each test substance provide challenges for development of in vitro approaches. The regional dosimetry and subsequent adverse outcomes are dependent on the physical state (gas, vapor, solid/liquid aerosol), characteristics and mechanism of action of the inhaled test substance; the accuracy of the potential to cause toxic effects these factors must inform the selection of the appropriate in vitro test system, exposure route, dosing paradigm and endpoints. We examined the acute exposure response of 3D human organotypic airway cultures grown at the air liquid interface (ALI) to the direct acting toxicant delivered either via direct pipette or liquid aerosol application. Our results underscore the need to (1) measure multiple endpoints to evaluate exposure-response profiles and 2) use deposited/absorbed dose and not exposure atmosphere concentration when assessing the toxicity of inhalable test materials using alternative in vitro test systems. While standardization of in vitro approaches is necessary to develop consistent data and progress toward regulatory acceptance, the limitations of these approaches for challenging chemistries such as viscous substances and those subject to hydrolysis in aqueous-based cell models must also be considered. To facilitate and inform selection of appropriate in vitro models, we have developed in silico approaches for assessment of inhaled toxicants that include the use of cheminformatics evaluation of the potential mechanism of action based on the molecular initiating and key events identified by the appropriate adverse outcome pathway (AOP). The goal of this integrated approach is to predict short- and long-term exposure values and to decrease need to conduct in vivo rodent inhalation toxicity studies.

Exposure to consumer products and related risk evaluations to avert safety issues is an important component to product development. The use of in silico approaches, coupled with human exposure data allows an estimation of realistic predictions of health effects that occur in a laboratory setting. With the ethical considerations of animal research, and in vivo
models poorly representing human exposure, human in vitro test systems are providing an avenue by which product developers can assess the potential adverse effects of the high number of product formulations requiring safety assessment. Fragrances are an integral component of many consumer products. As part of a joint project between the Research Institute for Fragrance Materials (RIFM) and Creme Global, a Monte Carlo model (called the Creme RIFM Aggregate Exposure Model) has been developed to estimate consumer exposure to ingredients in cosmetic, personal care products, and household and air care products. Using estimated exposure levels, in vitro exposure systems are utilized to deliver aerosolized materials to 3-dimensional tissues such as human reconstructed airways and precision-cut lung slices. Exposure dosimetry can be assessed using quantitation by quartz microbalances and quantities of test materials (as assessed using analytical means) in the exposure bays used for human tissue exposure. Relevant endpoints such as cytotoxicity and inflammatory responses can be used to better understand the potential effects of exposure to human airways at concentrations estimated from realistic exposure scenarios.

**1052 Nanoparticles and Microplastics Inhalation Studies: In Vivo to In Vitro Dose Extrapolation**

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Appropriate particle dose setting is needed for relevant in vitro studies. In vitro concentration should reflect the in vivo internal dose at the target organ burden. We used organ burden database of in vivo studies to calculate in vitro doses for lung and liver. We propose and exemplify a six-step approach for using nanomaterial in vivo organ burden data for in vitro dose setting: (1) Determine in vivo scenario to be reflected in vitro; (2) identify in vitro organ burden at (or close to) LOAEC; (3) extrapolate in vivo organ burden to in vitro effective dose; (4) extrapolate in vitro effective dose to in vivo nominal concentration; (5) set in vivo dose range around LOAEC-equivalent dose to allow establishing dose-response relationship; (6) consider uncertainties and identify specificities of in vitro test system potentially affecting in vivo findings. In vitro doses obtained with this approach are generally lower than those used in previously published in vitro studies. Selecting relevant in vivo doses will help designing in vitro studies and utilizing in vitro data for the assessment of particulate test materials.

**1053 Regulatory Acceptance of In Vitro Approaches to Evaluate Inhalation Exposure**


The Environmental Protection Agency’s (EPA) Office of Pesticide Programs (OPP) regulates the use of all pesticide chemicals. To evaluate potential risks to humans, the OPP evaluates exposures from multiple routes, including inhalation, as part of the human health risk assessment. Whole animal studies are typically required and/or used to evaluate inhalation exposures; however, regulatory statutes provide the EPA with the flexibility to modify the actual data and studies required on an individual chemical basis. Therefore, the Agency may use data from alternative methods and strategies to satisfy data requirements. OPP has developed guidance on waivers for in vivo subchronic inhalation studies, which uses a weight of evidence approach to consider physiochemical properties, toxicological profiles, exposure patterns, mode of action information, and structurally similar chemicals. Additionally, the EPA’s strategy to reduce animal testing relies heavily on the development and implementation of 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 chemical hazard and risk assessment. This presentation will discuss efforts to evaluate NAMs for inhalation risk assessments to support pesticide registrations, including the process taken thus far to develop and implement a NAM to refine inhalation risk assessment for a contact irritant using an in vitro assay derived from human airway epithelial cells.

**1054 Using Human Genetics to Aid in Safety Assessment of Therapeutics**

J. Yuan. Pfizer Inc., Cambridge, MA.

Preclinical safety evaluation of pharmaceuticals relies mainly on studies in animal models. While the use of animals has made significant contributions to safety assessment, there remains uncertainty in the extrapolation of risk from animals to humans. This is particularly true for novel therapeutics that do not cross-react to animal orthologs and lack pharmacological activity in species used for toxicology studies. Therefore, development of human-cen-
Drug-induced liver injury (DILI) is a serious safety concern with >1,000 drugs being reported to possess the potential to cause liver injury. However, most DILI occurs at low frequency (idiosyncratic) and some are genetically driven. Despite the vigorous and extensive safety testing during the drug development process, DILI remains an enigma. We have been developing the Liver Toxicity Knowledge Base (LTKB) to develop a content-rich resource to improve understanding of liver toxicity and ultimately for the FDA to utilize and reference when developing new therapies that have safety hazards through understanding who is at increased risk of experiencing adverse events and using genetic information to reduce risks. In this synopsis, we will summarize the state of the science to reaffirm the opportunities to impact drug discovery, development, and post-marketing pharmacovigilance, as well as to highlight the gaps in understanding and practice for these insights to benefit patients.

Observation is the starting point of discovery. Based on observations scientists form hypotheses that are then tested. In the information trillions of observations are being made and recorded every day – from online social interactions to the emergency room visit. With so much data available, generating hypotheses using a single scientist’s mind is no longer sufficient. Data mining is about training algorithms to recognize patterns in enormous sets of data and automatically identify new hypotheses. In this talk, I will discuss how we use data mining algorithms to characterize drug response in ancestrally diverse patient populations (NYC EHR data) and use large repositories of genetic data (both local and the UK Biobank) to identify genetic risk factors of adverse drug reactions. I will demonstrate how statistical machine learning can produce patient phenotypes even when primary diagnosis data is unavailable. This allows us to use noisy databases, like the electronic health records, for genetics and pharmacogenomics studies.

The COVID-19 pandemic is one of the most crucial global health calamities of the century spreading around the globe. This spread has been attributed to the transmissibility of the virus combined with scarcity of critical infrastructures and the unique challenges of sustaining population health and protecting the environment. The session is focused on the impact of COVID-19 (specifically toxicological, environmental, public health, and vulnerable indigenous populations) on the African continent given that the infrastructure and resources to deal with this calamity are extremely limited or are lacking in some countries. The community dynamics in various African localities also pose a great challenge to mitigate the threat of the virus to the 1.2 billion population of the continent. As of April 2020, greater than 140,000 confirmed cases in Africa are much lower than in the United States. The available data suggest that most African countries have inadequate health care systems and access to them, with challenges such as lack of equipment, funding, insufficient training of healthcare workers and inefficient data transmission. Even personal protective equipment (PPE) and basic hygienic supplies like soap and water are subject to shortages in some parts of the continent. Despite all these challenges, it is important to recognize that some progress is being made by some African countries. For example, scientists in Nigeria and Ghana have sequenced the SARS-CoV-2 genome, while Senegal and Nigeria have sequenced the SARS-CoV-2 genome and this calls for action in developing regional/global research strategies and collaborations.
The session speakers will present and discuss the spread and toxicological, environmental, and public health impacts of the COVID-19 pandemic outbreak in Africa, focusing on lessons learned, identifying research needs, and proposing research strategies for collaborative work and coordination of ongoing interventions to adequately address this global pandemic.

1063 Developmental Toxicity Hazard Assessment without Animals: Pathways and Prospects
J. M. Rogers, US EPA, Research Triangle Park, NC.

Global efforts are underway to transition toxicological risk assessment away from vertebrate animal models, as exemplified by the announcement from the US EPA administrator announcing the Agency’s intent to virtually eliminate use or funding of vertebrate animal research within 15 years. The Lautenberg Chemical Safety for the 21st Century Act requires that new approach methodologies (NAMs) be developed and that NAMs should replace animal models only when they are deemed to provide information of equivalent or better scientific quality. This goal is perhaps most aspirational for developmental toxicity hazard assessment given the complex and dynamic nature of human pregnancy and embryofetal development. “Teratogen screens” have been developed and used for prioritization and mechanistic studies for decades. Now, new discoveries and technologies, along with the urgency to reduce animal usage, have emboldened the belief that replacement of animals for developmental toxicity hazard assessment and dose-response is within sight. NAMs that are based largely on in vitro data and in silico models provide a path forward to animal-free testing for developmental toxicity. This session will present the latest in NAMs, including cheminformatics and connectivity mapping, utility of transcriptomics for hazard assessment, advances in stem cell models, and data driven in silico models. Current examples will be provided. Establishing scientific confidence in the reliability and relevance of NAMs requires comparisons to our current human and animal knowledge base, and automated tools to enhance literature searching and reference data curation/annotation will be discussed. The unifying theme of the session and the panel discussion to follow will be to discuss breakthroughs, opportunities, and challenges in NAMs for developmental toxicology, and how they can ultimately contribute to an animal-free system for developmental hazard identification and dose-response that is equivalent to or better than existing animal-based regulatory tests.

1064 Introduction: Evolution of Approaches to Animal-Free Predictive Developmental Toxicology: Can We Get There from Here?
J. M. Rogers, US EPA, Research Triangle Park, NC.

Since its beginning in the 1960s, developmental toxicity testing for regulatory decision-making has depended on mammalian laboratory species. Given the resources required for such studies, alternatives based on cells and tissues in vitro and “lower” species have for many years been developed to gauge comparative toxicity and for mechanistic understanding. The totality of this in vivo and in vitro database along with the embryological knowledge, technological advances and computational capabilities that have been gained allow a vision of animal-free developmental hazard assessment to begin coming into focus. This introduction will provide a brief history of where we’ve been, where we are, and the multiple paths leading to a destination that will be envisioned in this session. An overview of the session will be provided, along with the charge to the speakers, thought leaders in the field, to give their vision of what developmental toxicity testing will look like in fifteen years.

Disclaimer: does not necessarily reflect US EPA policy.

1065 Biotechnology Plus 60 Years of Data Support Predictive Toxicology
G. Daston, Procter & Gamble, Cincinnati, OH.

Systematic organization and review of toxicity testing reveals that there are more than 30,000 DART studies on more than 20,000 distinct chemical entities. Cheminformatic analysis of these studies, along with more basic research on molecular targets and mechanisms of toxicity, allow us to define the known universe of modes of action for developmental toxicity. This then allows us to query chemical activity by mode of action (as revealed by global gene expression analysis and mechanistic assays) to infer toxicity and potency. Toxicology predictions are possible now using this approach, combined with read-across, and with time will be possible for novel chemistry and mixtures where read-across is not useful.

1066 Automated Approaches to Anchoring Alternatives
N. Kleinstreuer, NIEHS/NICEATM, Research Triangle Park, NC.

Establishing confidence in New Approach Methodologies (NAMs) and working towards the ultimate goal of replacement requires curating and annotation of high-quality in vivo studies as well as a deeper understanding of the limitations of reference animal data to set appropriate performance expectations. Advanced computational capabilities can automate the processes of identifying and collecting information from toxicology studies, which have traditionally required manually intensive efforts. Natural language processing (NLP) approaches, paired with artificial intelligence (AI) and machine learning, are being leveraged to identify high quality developmental toxicity studies in the scientific literature based on prenatal study protocol minimum criteria from regulatory guidelines. Apical effect extractions from National Toxicology Program developmental toxicity studies and high-quality studies submitted to the European Chemicals Agency were programmatically annotated using a cross-walk of three controlled vocabularies and ontologies, resulting in >30,000 rows of coded data. Structured and annotated databases support characterization of additional reference chemicals with fetal-specific or sensitive effects, identification of endpoint prevalence, and correlation with chemical attributes such as structural features and mechanistic bioactivity patterns. These curated reference data function as anchors when establishing scientific confidence in alternatives. Examples of integrative analyses to determine the contributions of NAMs (e.g. stem-cell platforms, QSAR models) to predict chemical developmental toxicity potential within the context of these annotated reference sets will be discussed. Computational tools such as AI, NLP, and machine learning can help not only evaluate NAMs and establish confidence, but also provide researchers with more streamlined access to comparable in vivo/in vitro studies.

1067 Stem Cell–Based In Vitro Morphogenesis Models to Investigate Developmental Toxicity of Chemical Exposures
Y. Marikawa. University of Hawaii, Honolulu, HI. Sponsor: J. Rogers

To reduce animal usage in developmental toxicology, pluripotent stem cells have been explored as in vitro models of early embryos to investigate teratogenic exposures. With recent advancements in cell culture methodology, three-dimensional aggregates of mouse and human pluripotent stem cells (referred to as embryoid bodies) can exhibit elongation morphogenesis, which recapitulate gastrulation to generate the germ layers and cranio-caudal body axis. Morphogenesis of embryoid bodies, as assessed by their growth and shape changes, and spatiotemporal expression patterns of developmental regulatory genes, are sensitively altered by various teratogenic exposures. Thus, morphogenetic embryoid bodies may serve as in vitro tools for the detection of potential teratogens. Furthermore, because stem cells are amenable to various molecular manipulations, morphogenetic embryoid bodies may provide valuable insights into the mechanisms of teratogenic action. These studies should continue to on-going efforts to construct Adverse Outcome Pathways for developmental toxicology. While morphogenetic embryoid bodies are promising tools, they have several limitations. For example, their current formats represent only early developmental stages, which may preclude the detection of teratogens that specifically affect events of later developmental stages. Also, in vitro models, including embryoid bodies, are generally devoid of the maternal environment, which is often responsible for metabolic conversions of chemicals into teratogenic forms. Such “pro-teratogens” may not be detected with embryoid bodies. These limitations need to be overcome by modifications of culture methods or collaborations with other types of in vitro models, so that stem cell-based morphogenesis models can be effectively incorporated in non-animal alternatives to screen a wide range of teratogenic exposures for regulatory purpose.

1068 The Application of Toxicogenomics in Adverse Outcome Pathway-Based Alternative Testing Strategies

Adverse Outcome Pathways (AOPs) facilitate the development of alternative testing strategies and provide structured representation of causally linked events at different levels of biological organization that lead to adverse health outcomes. The use of genomic approaches in toxicological studies has vastly improved our ability to describe global molecular perturbations that precede and correlate with chemical toxicity. Coinciding with the wide availability of public toxicogenomic datasets, methods are emerging to coalesce data to determine conserved toxicological signatures across in vivo and in vitro...
model systems. We propose that the application of integrated toxicogenomic profiles may inform and enhance AOP design and hazard identification. To demonstrate the value of this approach, we performed a case study of all-trans retinoic acid (RA) signaling and developmental neurotoxicity (DNT). First, we summarized evidence to outline a provisional AOP related to altered RA signaling and adverse CNS developmental outcomes. Next, we defined a relevant RA-exposure scenario with RA-induced DNT (RA-DNT) conserved across human, rodent, and zebrafish models. Finally, we utilized the RA-DNT signature to identify specific antigens (azoles) that perturb RA signaling, and potentially, cause DNT during vulnerable windows in early CNS development. In summary, our study highlights the utility of toxicogenomic approaches in AOP-based testing strategies aimed at identifying chemical hazards. This work was kindly supported by Organisation for Economic Co-operation and Development (OECD) and the National Institute of Environmental Health Sciences (NIEHS) (R00ES023846).

1069 Synthetic Microsystems, Computational Intelligence, and Artificial Life
T. B. Knudsen, US EPA, Research Triangle Park, NC.

New Approach Methodologies (NAMs) that are based largely on in vitro data and in silico models provide a path forward to animal-free testing of the potential for developmental and reproductive toxicity (predictive DART). Data-driven and embryologically-inspired in silico models are both needed for the synthesis and integration of complex in vitro data with vast understanding of developmental processes and toxicities in vivo. This can be achieved with synthetic microsystems that combine the in vitro potential of biomimetic embryology with morphogenetic computer models that capture the cellular dynamics of developmental systems. For example, high-throughput datasets generated with embryonic stem cell (ESC) assays provide a path forward for data-drive models in predictive DART. Placing these effects in the context of in vivo biology is a challenge. The epiblast is the germ layer origin for most cell types of the embryo proper and harbors the embryology fundamentally recapitulated by the ESC assay. Like pluripotent ESCs, the epiblast is autopoietic (self-organizing) pluripotent cells in the epiblast retain positional information critical for embryonic patterning. The genomic blueprint of the embryonic body plan is then decoded gastrulation. Conserved cell signaling pathways (e.g., WNT, FGF, BMP, NODAL, ATRA, ~) establish polarity and axial asymmetry via the primitive streak and in mammals are set up by extramembranous and uterine factors. Recently developed ‘synthetic epiblast’ microsystems using hESCs/iPSCs, microfluidics and bioprinting can be used to artificially impose extrinsic gradients and investigate mechanisms by which the human body plan unfolds. Computational models armed with sufficient intelligence of cellular behaviors and relationships can recapitulate regional specification of the early embryo during gastrulation. Simulation of ‘quasi-gastrulation’ with artificial life complements in vitro data-driven models, putting into cellular motion how chemicals affecting ESC behaviors might disrupt the embryo under different exposure scenarios. Artificial life may, in this manner, contribute animal-free platforms for developmental hazard identification and dose-response modeling. Disclaimer: does not necessarily reflect Agency policy.

1070 From Inhaled Particles to Neurodegeneration and Toxicity: Evidence from Studies in Volunteers, Experimental Animals, and Cell-Based Systems
F. R. Cassee, Rijksinstituut voor Volksgezondheid en Milieu (RIVM), Bilthoven, Netherlands.

A rich body of literature exists that has demonstrated adverse human health effects on the brain following exposure to ambient air particulate matter, and there is strong support for an important role of ultrafine (nanosized) particles, mainly from combustion processes. With global production having increased exponentially over the past decades, and a significant proportion not being disposed of properly, engineered and plastic particle pollution also is now a serious concern for brain health. Plastic debris spans orders of magnitudes in size, including nanoplastics, originating from products within which they were intentionally added, such as cosmetics or generated during use or by the degradation of larger plastic items, such as textiles, tire wear, or artificial turf. Toxic effects are specifically designed and used very small, also with a wide range of applications, including in food and medicines. A key feature of such ultrafine/nanosized particles is their ability to cross biological barriers and thereby reach other organs than the lung, as well as the ability to be taken up via olfactory and trigeminal nerves, thereby bypassing the blood-brain barrier, leading to neurotoxic effects following inhalation exposures. This Symposium will highlight our current understanding of nanoparticle-induced neurotoxicity from various sources of emissions.

1071 From Ambient Particulate Matter, to Nanomaterials, to Microplastics—Similarities and Differences with Respect to Dosimetry and Toxicity: Introduction
F. R. Cassee, Rijksinstituut voor Volksgezondheid en Milieu (RIVM), Bilthoven, Netherlands.

Similar effects as has been shown for ambient ultrafine particles as well as engineered nanomaterials and nanosized plastics can be expected based on their poor solubility and persistence in the body. At present their effects on human health are unclear, including their adsorption, biodistribution, and potential toxic effects, and neither is their ability to translocate across biological barriers and reaching secondary organs clear, nor what extent, how and when this may lead to adverse health effects. What can we learn from existing information on ultrafine particle pollution to predict effects of the advanced nanomaterials and small plastic particles? Similarities and differences between the various particle group will be discussed as introduction to more in-depth presentations. Specific emphasis will be put on the dosimetry and biodistribution of nanomaterials.

1072 Developmental Neurotoxicity of Traffic-Related Air Pollution
T. B. Cole, University of Washington, Seattle, WA.

Air pollution is an important contributor to the global burden of disease, particularly to respiratory and cardiovascular diseases. In recent years, evidence is accumulating that air pollution may adversely affect the nervous system as shown by human epidemiological studies and by animal models. Age appears to play a relevant role in air pollution-induced neurotoxicity, with growing evidence suggesting that air pollution may contribute to neurodevelopmental and neurodegenerative diseases. Ultrafine particles (UFP) emitted by traffic (e.g., diesel exhaust) are an important contributor to urban air pollution, and may possibly be its more relevant component than larger sized, regulated particulate matter. Air pollution is associated with increased oxidative stress and inflammation both in the periphery and in the nervous system, and UFP can directly access the central nervous system. This presentation focuses on the adverse effects of UFP on the developing brain; it discusses some characteristics that make the developing brain more susceptible to toxic effects, and summarizes the animal and human evidence suggesting that exposure to elevated air pollution is associated with a number of behavioral and biochemical adverse effects. It will also discuss in more detail the emerging evidence of an association between perinatal exposure to UFP and increased risk of autism spectrum disorder (ASD). Some of the common mechanisms that may underlie the neurotoxicity and developmental neurotoxicity of UFP are also discussed. Findings will be presented on an experimental model in mice exposed to diesel exhaust during prenatal development and early postnatally.

1073 Brain Metal Dyshomeostasis and Ultrafine Particulate Matter and Neurodevelopmental Disorders
D. A. Cory-Slechta, University of Rochester Medical Center, Rochester, NY.

Epidemiological studies have associated air pollution (AP) exposures with multiple neurodevelopmental disorders (NDDs), including autism spectrum disorder, schizophrenia and attention deficit hyperactivity disorder, all of which are male-biased. While each of these NDDs has unique features, they also share multiple characteristics which are replicated in our studies in mice of early postnatal (human 3rd trimester brain development equivalent) exposures to ambient ultrafine particles (UFPs). Considering the most reactive component of AP. Such exposures resulted in male-biased and persistent ventriculomegaly (enlarged lateral ventricles), microglial activation, elevated glutamate levels, reduction in size and myelination of the corpus callosum, and nucleus accumbens cell death; behavioral features have included impaired social behaviors and increased impulsivity. Collectively these findings provide biological plausibility for the epidemiological associations of AP with NDDs. In addition, developmental UFP exposures resulted in marked elevations of metals in brain, including iron, copper and aluminum. Excess of brain metals can result in brain metal dyshomeostasis, impairing brain development and function. Consequently, a subsequent study of early postnatal exposures to Fe nanoparticles, in conjunction with SO2 (a component of AP that facilitates Fe bio-solubility and uptake), likewise produced male-biased ventriculomegaly and nucleus accumbens cell death, consistent with effects of ambient UFPs. A role of AP-induced brain trace metal dyshomeostasis could also underlie the male bias in NDDs based on a higher population of colonizing microglia in male brain at the time of exposures as microglia and iron are highly interactive. It is notable that brain trace metal dyshomeostasis,
and particularly elevated iron, are also seen in neurodegenerative disorders. Collectively, these findings suggest that AP could be a broad risk factor for NDDs and thus understanding component(s) of AP that underlie these effects is critical to understanding mechanisms, defining potential intervention strategies and protecting public health via AP regulation.

1074 Traffic-Related Air Pollution Particles: A Risk Factor for Alzheimer’s Disease?
R. P. Schins, IUF—Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany. Sponsor: F. R. Cassee.

There has been increasing concern that inhalation of ultrafine particles (UFPs), as part of the air pollution mixtures, contribute to Alzheimer’s disease (AD) pathogenesis. The design of our study consisted of controlled inhalation exposures of micro- and nanoplastic model particles mixtures and particular (model) particles or mixtures have contributed to elucidation of mechanism whereby particles may contribute to development of diseases. However, real world exposures such as performed in our study offer a better alignment with epidemiological studies and thus link these observational studies in humans with controlled animal experimental approaches. The results from such “on-site” inhalation exposure studies demonstrate, to what extent, and how, traffic-related particles contribute to AD-pathogenesis in rodent inhalation toxicology models, adding vital mechanistic support to the epidemiological findings. This will be further illustrated with data on engineered nanomaterials. The results of our investigations contribute to the growing debate on the question if nanosized insoluble particles including those emitted by traffic or as plastic pollution can be considered as novel AD risk factor.

1075 Exposure to Inhaled Incidental Nanosized Particles Activates Markers of Neurotoxicity and Alters Dopaminergic Transmission
A. De Vizcaya-Ruiz, Cinvestav, Mexico City, Mexico.

Exposure to incidental particles as those generated as air pollutants, fine and ultrafine (nanosized) particles (FP and UFP), has been associated with increased risk of neurodegenerative diseases (i.e. Parkinson’s disease), depression and schizophrenia; disorders also related with altered dopaminergic transmission. Exposure of animal models (male Sprague-Dawley rats) to FP and UFP confirmed a higher induction of oxidative stress and inflammation (HO-1, SOD-2, IL-1ß and TNFa increased expression) by UFP in comparison to FP and the striatum as a target site for particle toxicity. The striatum, a neuronal nucleus with extensive dopaminergic innervation, also showed astrocyte activation and modifications in dopamine content and D2 receptor (D2R) density as a consequence of particle stimulation. Using in vitro models, namely rat striatal isolated nerve terminals (synaptosomes) and native D2Rs and cloned human D2Rs (expressed in CHO-K1 cell line) an alteration of dopamine uptake and release, and D2R affinity and signaling occurred, confirming direct dopamine transmission effects induced by exposure to UFP.

1076 In Vitro Neurotoxic Hazard Characterization of Micro- and Nanoplastics
R. Westerink, Universiteit Utrecht, Utrecht, Netherlands.

Given the global abundance and environmental persistence, human exposure to micro- and nanoplastics (MNPs) is unavoidable. Humans are predominantly exposed via consumption of food and consumer products. Recently it became evident that exposure also occurs via consumption of air as MNPs are released from textiles, synthetic rubber tires and plastic covers. It has also become demonstrated that nano-sized particles such as gold (Au) and titanium dioxide (TiO2) nanoparticles, can reach the brain after inhalation to exert a range of neurotoxic effects. Current evidence regarding uptake, translocation and the potential neurotoxicity of MNPs is scarce. We therefore investigated acute (30 min) and repeated (21 days) exposure effects of different MNPs on neuronal activity of rat primary cortical cultures grown on micro electrode arrays (MEAs). While the results suggest limited effects on neuronal activity and cell viability, parallel experiments demonstrated that exposure to MNPs can result in inhibition of acetylcholinesterase activity in vitro. To increase the relevance of this in vitro neurotoxic hazard characterization, the ability of in particular nanoplastics to cross an in vitro blood-brain barrier model examined, indicating that nanoplastics can actually reach the brain upon systemic uptake. This was confirmed in a proof-of-principle study in which mice were orally exposed to microplastics for one to ten days. Microscopy analysis revealed that also in vivo small plastic particles have the intrinsic ability to reach the brain. Given the ability of small plastic particles to reach the brain, a systematic comparison of the neurotoxic effects of different particle types, shapes, sizes at different exposure concentrations and durations is urgently needed to further elucidate the neurotoxic hazard and risk of exposure to MNPs.

1077 Novel Emerging Treatments for Acetaminophen Toxicity
J. Dear, University of Edinburgh, Edinburgh, United Kingdom.

Acetaminophen (paracetamol, N-acetyl-p-aminophenol; APAP) is the most common drug taken in overdose in the US and United Kingdom (UK). In the US, APAP overdose accounts for more than 56,000 hospital visits and around 450 deaths due to acute liver failure each year. Annually, overdose directly leads to around 100,000 hospital visits in the UK, with around half of these patients admitted to hospital for emergency antidote treatment. APAP also is directly responsible for the deaths of 100-150 people per year in the UK. The mechanism of APAP hepatotoxicity has been extensively investigated in mouse models that faithfully represent the human disease and in human hepatocytes. At therapeutic doses, APAP is mostly glucuronidated and sulfated, then excreted. A small percentage is converted to the reactive intermediate N-acetyl-p- benzoquinoneimine (NAPQI), which is detoxified by reaction with glutathione. However, after overdose, excess NAPQI binds to intracellular proteins, causing increased oxidative stress, mitochondrial injury, and cell death. N-acetylcysteine (NAC) is the current treatment for APAP overdose. It acts by replenishing hepatocellular glutathione to increase the detoxification of NAPQI. Although highly effective at preventing hepatotoxicity when used within 8 h of overdose, it is associated with the following challenges: (1) reduced efficacy when administered later than around 8 h after overdose ingestion; (2) adverse drug reactions (ADRs): nausea/vomiting occurs in more than half of recipients and anaphylactoid reactions in about a third; (3) prolonged duration: the regime is time-consuming, taking at least 21 h, leading to significant hospital bed occupancy. Recently, much-needed new treatments for APAP overdose have emerged, guided by increased understanding of the underlying pathophysiology and galvanized by new high sensitivity/specificity biomarkers of liver injury. In this session, we will review the landscape of new treatments with presentations from world-leading preclinical and clinical experts who are leading these efforts.

1078 What Does a New Treatment for Acetaminophen Overdose Have to Be Able to Do?
R. Dart, Rocky Mountain Poison and Drug Center, Denver, CO. Sponsor: J. Dear.

Acetaminophen (APAP) overdose is the primary cause of acute liver failure and related deaths throughout the USA, UK, and several other countries. The standard-of-care treatment is N-acetylcysteine (NAC); however, NAC loses efficacy >8 h after APAP overdose. Beyond that, a liver transplant may be the only option. In this presentation, Dr. Dart will discuss the patient journey from taking an APAP overdose; through clinical presentation, emergency treatment, and development of acute liver injury and acute liver failure; to resolution of injury and discharge from hospital. Along the way, he will identify key inflection points where an effective new treatment could have a significant impact on patient care and outcomes.

1079 Novel Regimens for Acetylcysteine Treatment
S. Thomas. Newcastle University, Newcastle, United Kingdom. Sponsor: J. Dear.

The only current licensed treatment for acetaminophen (APAP) overdose is N-acetylcysteine (NAC). It is highly effective if started soon after overdose (within approximately 8 hours), but efficacy subsequently decreases with time. Furthermore, NAC commonly causes dose-related adverse effects, such as nausea. Over the last decade new regimens for delivering NAC have been developed. Clinical trials have demonstrated that these regimens are associated with reductions in adverse effects and are potentially shorter to administer, without a reduction in effectiveness with regard to preventing liver injury. They offer the prospect of safer and better tolerated therapy for patients at low risk from acetaminophen poisoning, as well as the promise of earlier hospital discharge. In this presentation, Prof. Thomas will give an overview of how NAC can be administered to optimise its risk/benefit profile based on data from recent clinical trials.
Lever injury and acute liver failure are serious clinical problems after acetaminophen (APAP) overdose in the western world. Early studies in mice recognized that an APAP overdose causes formation of a reactive metabolite, which depletes hepatic glutathione and binds to cellular proteins. This provided the rationale for the rapid testing of N-acetylcysteine (NAC), a drug already on the market for treatment of lung diseases, in patient trials and the accelerated regulatory approval for use in APAP overdose patients. NAC, which supports the recovery of hepatic GSH levels and thus facilitates the scavenging of the reactive metabolite and of reactive oxygen species, is highly effective when administered within 8 h after the overdose. However, the efficacy of NAC is limited in patients presenting late and in patients with a very severe overdose. In addition, NAC treatment can have side effects (e.g. anaphylactic reactions). Nevertheless, even 40 years later, NAC is still the only clinically approved antidote against APAP hepatotoxicity. To address that, during the last 10 years, significant progress has been made in more detailed understanding of the different phases of APAP-induced liver injury in the murine model and the translation of these mechanisms to the human pathophysiology based on studies in primary human hepatocytes and use of mechanistic biomarkers in APAP overdose patients. From these studies, a number of therapeutically relevant targets were identified including cytochrome P450 enzymes, c-Jun N-terminal kinases (JNK) and mitochondrial oxidant stress and peroxynitrite formation. These findings led to the identification of 4-methylpyrazole (4MP) as potent inhibitor of both cytochrome P450 and of JNK resulting in effective protection against APAP toxicity after early and late treatment. In addition, studies with the mitochondria-targeted SOD mimetic Mito-Tempo provided the rationale for the development of the SOD mimetic calmangafodipir. Based on these studies in clinically relevant animal models and human hepatocytes, it is expected that these new drugs can complement NAC as antidotes for APAP hepatotoxicity or even expand its therapeutic window.

Clinical Studies with Fomepizole in Acetaminophen Overdose

S. C. Curry, University of Arizona, Phoenix, AZ. Sponsor: J. Dear

4-methylpyrazole (4MP; fomepizole) is known to reduce acetaminophen (APAP)-induced liver injury in rodent models through inhibition of cytochrome P450 enzymes, as well as altered c-Jun N-terminal kinase (JNK) signaling. Several case reports have provided supporting evidence for use of 4MP to treat APAP overdose in humans as well, but until recently no controlled clinical trials of 4MP in human APAP toxicity had been performed. Here, Dr. Curry will discuss the history of 4MP as a potential treatment for APAP overdose, and present results from a crossover trial of 4MP in healthy human volunteers after ingestion of a large sub-toxic dose of APAP to describe 4MP’s effect on APAP metabolism in humans.

Clinical Studies with Calmangafodipir in Acetaminophen Overdose

J. Dear, University of Edinburgh, Edinburgh, United Kingdom.

Calmangafodipir is a first-in-class superoxide dismutase mimetic that prevents acetaminophen (APAP) toxicity in mice. To take calmangafodipir from pre-clinical development into patients, the POP Trial was performed. The POP Trial was a phase 1, open label, rising dose, randomised study which explored the safety and tolerability of calmangafodipir co-treatment with a 2 bag, 12 h, N-acetylcysteine (NAC) regimen in APAP overdose patients. The primary outcome was the safety and tolerability of calmangafodipir combined with NAC. The POP Trial reported that calmangafodipir was well tolerated in patients treated with NAC for APAP overdose. The pivotal phase II/III study is planned to be initiated in 2021. In this final talk, Prof. Dear will discuss the challenges of drug development in clinical toxicology and the potential utility of calmangafodipir to treatment APAP overdose patients.

The State of CRISPR-Cas9 Gene-Editing Technology and Applications for Toxicology Research

L. Wehmas, US EPA, Research Triangle Park, NC.

The gene editing technology Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 is revolutionizing basic biology, translational medicine, and increasingly, the field of toxicology. CRISPR-Cas9 silences or overexpress targets through non-homologous end joining or homology directed repair making it uniquely suited to facilitate adverse outcome pathway discovery and development. The roles of particular target molecules in pathways of toxicity can be functionally verified and linked to actionable regulatory outcomes with this technique pushing beyond associations to demonstrate mechanistic causality. This innovative technology has critical ramifications for hazard identification, chemical prioritization, risk assessment, and drug discovery. Gene editing can be conducted in a variety of cell types and animal models allowing for the transition of results across complex biological systems via validation of conserved toxicity pathways. The availability of many different guide RNA design strategies and genome wide knockout libraries allow for individual, multi, and genome-wide gene edits for positive and negative selection-based screening assays, while application of CRISPR-deactivating Cas9 can illuminate site-specific methylation/acetylation changes necessary to characterize chemical mediated epigenetic modes of toxicity. While most gene editing applications have focused on loss-of-function strategies to understand key toxicity events, homology directed repair can be used with CRISPR to insert or swap out gene targets for tailored gain-of-function mutations. This can not only assist in recapitulating a specific adverse outcome pathway or disease state, but also be coupled with single base pair editing to understand how common mutations in sensitive subpopulations impact chemical toxicant susceptibility. This introduction will present the state-of-the-science on CRISPR-Cas9 gene editing while discussing applications, advances, and the ramifications of this technology on the field of toxicology. This abstract does not represent US EPA policy.

Functional Pathway Identification with CRISPR-Cas9 Genome-Wide Knockout Screening in Human Dopaminergic Neuronal Cells following Chronic Treatment with Dieldrin or Iron


Parkinson’s disease (PD) is the second most common neurodegenerative disorder in the US and worldwide with most cases presenting late in life and of idiopathic origin. The degeneration of dopaminergic neurons in the substantia nigra is a hallmark of PD and studies have illustrated that dieldrin or iron toxicity may lead to the loss of dopaminergic neurons. However, the pertinent
mechanisms of action of both dieldrin and iron on dopaminergic neuronal toxicity remain poorly understood. In this study, we carried out a genome wide (Brunello library, 19,114 genes, 76,411 sgRNA) CRISPR-Cas9 screen in human dopaminergic SH-SY5Y neuronal cells chronically exposed (30 days) to dieldrin or ferric citrate to identify cellular pathways that are functionally related to cellular toxicity. Our results indicate that dieldrin toxicity was enhanced by genetic disruption of specific components of the ubiquitin proteasome system (UPS) as well as the protein degradation pathways previously implicated in inherited forms of PD, centered on parkin, the ubiquitin E3 ligase. Additionally, disruption of regulatory components of the mTOR pathway which integrates cellular responses to both intracellular and extracellular signals and is a central regulator for cell metabolism, growth, proliferation and survival, led to increased sensitivity to dieldrin-induced cellular toxicity. Not unexpectedly, disruption of iron storage genes enhanced the cellular toxicity of iron. Similar to dieldrin, components of the UPS were important for cellular resistance to iron, but the specific subset of UPS components was distinct. Disruption of mTOR signaling also modulated iron toxicity which supports a common role for mTOR in cellular response to stress. Interestingly, loss of a potassium channel, KTCD5, with homology to the Drosophila melanogaster insomniac locus enhanced the cellular toxicity of iron. This study was one of the first to apply a genome wide CRISPR/Cas9-based functional gene disruption screening approach in an adherent neuronal cell line to globally decipher cellular mechanisms that contribute to environmental toxicant-induced neurotoxicity and, in doing so, provides novel insight into the neurotoxicity associated with chronic exposure to dieldrin or iron.

1087 Using CRISPR-Cas to Identify How Endocrine Disruptors Cause Malformations and Functional Defects in the Zebrafish Heart

D. Gorelick, Baylor College of Medicine, Houston, TX.

Environmental endocrine disruptors are chemicals in the environment that mimic endogenous hormones or influence their synthesis and metabolism. Estrogens and progestins are two types of endocrine disruptors that can have effects on organ systems throughout the body by binding to estrogen and progestrone receptors. Because the same chemical can bind to multiple receptors, it has been difficult to identify the receptor that mediates toxic phenotypes, particularly when animals are exposed to chemical mixtures. Here we show that exposing zebrafish embryos to estrogens and progestins is associated with abnormal heart development, and that these chemicals act via membrane-associated hormone receptors. To determine the causative receptors, we used CRISPR-Cas technology to generate zebrafish strains with mutations in each sex hormone receptor. We found that 17beta-estradiol, a potent phallogenic estrogen, increased embryonic heart rate via G protein-coupled estrogen receptor 40, independent of nuclear estrogen receptors alpha and beta. Exposure to Primodos, a hormone-based pregnancy test used in the 1960s and 1970s composed of the progestin norethindrone and the estrogen ethynylestradiol, caused cardiac edema and abnormal heart loop-
Cytochrome P450 (P450, CYP) enzymes have long been of interest due to their roles in the metabolism of drugs, pesticides, pro-carcinogens, and other xenobiotic chemicals, as well as their critical roles in the biosynthesis and metabolism of steroids, vitamins, and certain eicosanoids. CYP enzymes play a dominant role in the activation of chemicals, especially potential carcinogens. This talk will highlight the current state of knowledge of ‘orphan’ P450s, as well as basic functional aspects, and address the practical applications of structural work, polymorphisms, and function in toxicology, new drug development, and targeted personalized medicine.

1091 High-Throughput Screening for Zebrasfish Cytochrome P450 Substrates Provides Insight into Enzyme Function

J. Wilson, McMaster University, Hamilton, ON, Canada.

The functions of many mammalian cytochrome P450 (CYP) enzymes are well understood and there are specific and selective probe substrates to assess enzyme expression and function. Yet, there are significant numbers of orphan CYP enzymes and functional characterization of these proteins is very challenging. These challenges are due to complex evolution and gene duplications in the families responsible for chemical detoxification and co-expression of CYP enzymes in detoxification organs. While heterologous expression of individual CYP enzymes proffers the assessment of individual CYP in vitro, deploying individual assays for compound metabolism is a slow and arduous process to characterize function. High-throughput screening offers an exciting opportunity to more quickly provide data on CYP mediated compound metabolism. In this talk, I will outline the major issues with assessing CYP mediated compound metabolism, based on evolutionary differences in gene complement and the overlapping capacities of zebrafish enzymes for fluorogenic probe substrates, using CYP1 and CYP3 enzymes as primary examples. To address these issues, we have developed a high-throughput screening approach based on the consumption of the co-factor nicotinamide adenine dinucleotide phosphate (NADPH) as an endpoint to indirectly measure catalytic activity. Using heterologously expressed enzymes and a 4000 compound library of pharmacologically active, natural or off-patent small-molecule compounds, we have screened zebrafish CYP1 and CYP3 enzymes. We observe variable hit rates, ranging from approximately 8% for CYP1A to 2.5% for CYP3A. The screen and follow up provide key data to assess the important role of CYPs in xenobiotic metabolism and a direct assessment of the potential for neo- or sub-functionalization of CYP enzymes.

1092 Cytochrome P450 Knockout Zebrafish in Toxicology

J. Goldstone, Woods Hole Oceanographic Institution, Woods Hole, MA.

Zebrafish are important test organisms for mechanistic toxicological research and for the safety assessment of manufactured and environmental chemicals, yet aspects of metabolism critical to the use of this model are not fully understood. Zebrafish are used in a regulatory context for environmental toxicology in Europe, and have become an important toxicity model in the US. However, while cytochrome P450 gene regulation is an important bioindicator in zebrafish, the functioning of the cytochrome P450 systems in xenobiotic metabolism is not well described. Gene-engineered model organisms offer potent systems for studying metabolism and disposition. We have produced and partially characterized transgenic knockout zebrasfish for five CYP1 genes, as well as for the orphan CYP20A1 gene. Knockout strains exhibit generally normal development, suggesting that endogenous substrates of these P450s may be generated by other P450s (substrate overlap) or may not be essential for laboratory reared zebrafish. Xenobiotic and indicator substrate metabolism is also altered in these knockout strains. In particular CYP1A1-null zebrafish larvae exhibit strongly decreased whole-body ethoxy- and methoxyresorufin (EROD and MROD) metabolism when treated with beta-naphthflavone for 24 hours, although the EROD/MROD ratio is nearly the same. In addition, the ‘orphan’ CYP20A1-null zebrafish exhibits altered behavior in different behavioral paradigms, particularly those correlating with hyperactivity. Zebrafish increasingly are being used as predictive models of toxicity in various organs, with predictivity in the range of 70-91%. This research provides a mechanistic understanding of CYP function in a powerful in vivo model.

1093 Transgenic Caenorhabditis elegans Models for Studying P450-Mediated Drug- and Toxicant-Induced Toxicity

J. Hartman, Duke University, Durham, NC.

Cytochrome P450 enzymes metabolize hydrophobic drugs, pollutants, and endogenous chemicals by a monooxygenase reaction that can result in detoxification, or, paradoxically, bioactivation to reactive metabolites. Although the metabolism of many chemicals by P450s has been studied in-depth through in vitro and in vivo experiments, many more chemicals’ toxicity and, importantly, mechanism of toxicity are not well understood. As a new tool to study the consequences of P450 metabolism, we developed transgenic Caenorhabditis elegans nematodes that express human cytochrome P450 enzymes or orthologs. C. elegans have 84 cytochrome P450 genes in their genome; however, total P450 expression levels and activity for common P450 marker substrates are very low relative to high-expression vertebrate tissues such as liver, giving a minimal background in these transgenic animals. The animals are also short-lived (with median lifespan about 17 days), allowing toxicity outcomes to be measured throughout their lifetime. Other benefits of C. elegans include a fully sequenced and well-annotated genome, access to genetic mutants and/or RNAi knockdown of most genes in the genome, ease of generating transgenic mutants through various methods including CRISPR/Cas9, and availability of many fluorescent reporters that allow for medium-throughput analysis of gene expression, redox status, and other measures. So far, we have successfully generated nematodes expressing zebrafish CYP1A1 and human CYP2E1. We have measured expression of these enzymes at the mRNA and protein level, and enzymatic activity using EROD and 4-nitrophenol assays, respectively. We cannot detect either enzyme expression or activity in wild-type animals but are able to measure robust expression and activity in transgenic animals. Compared to wild-type, transgenic CYP1A-expressing nematodes are dramatically protected from PAH toxicity, while CYP2E1-expressing animals are more sensitive to toxicity from the drug acetaminophen. In ongoing and future studies, we are using CRISPR/Cas9 to test whether loss or inhibition of CYP1A1 and CYP2E1-expressing animals, creating a CYP1B1-expressing nematode, as well as targeting expression of these P450 enzymes specifically in cells of interest, such as dopaminergic neurons. This model holds promise for mechanistic toxicological studies of compounds’ which are metabolized through cytochrome P450 enzymes.

1094 Novel Knockout Animal Models for Studying P450-Mediated Toxicity

W. Baldwin, Clemson University, Clemson, SC.

Our laboratory is investigating the roles of CYPs in the regulation of lipid metabolism, obesity, and liver disease in rodent models, and the mechanisms by which toxicants such as PFOS disrupt lipid homeostasis. This presentation will include discussion of our current research related to the potential for cytochrome P450 (CYP) inhibition, specifically the inhibition of Cyp3a and Cyp2b2 members by environmental toxicants, as a mechanism for obesity, fatty liver disease, and other metabolic disorders. We have recently developed a novel knockout mouse model using Crispr/Cas9 to test whether loss of inhibition of Cyp3a increases the likelihood that individuals will develop non-alcoholic fatty liver disease (NAFLD) and obesity. We will also present novel research evaluating how environmental pollutants may inhibit Cyp2b or Cyp3a enzymes and may increase NAFLD and obesity during treatment with normal or high-fat diets. Overall, the purpose of this project is to test whether alterations in Cyp2b and Cyp3a activity (such as chemical inhibition) can alter the allocation of fatty acids and in turn cause NAFLD and obesity.

1095 New Approach Methodologies for Exposure: Advancing Chemical Risk Assessment

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

This Workshop will review new approach methodologies (NAMs) for exposure science that have been developed to address the thousands of chemicals in commerce and the environment with little or no data. Public health chemical challenges and chemical regulation. These chemicals include those present in consumer product ingredients). NAMs for exposure science include (1) new methods that can be broadly applied for biomonitoring and biostatistics, (2) the
application of machine learning to data-poor situations, (3) high-throughput exposure models for making predictions from limited chemical and scenario descriptors, (4) high-throughput toxicokinetic data and models enabling in vitro to in vivo extrapolation (IVIVE) of high-throughput toxicity data, and (5) chemical risk prioritization strategies that can be broadly applied across large numbers of chemicals. This Workshop will review new technological developments, including exposure science modeling approaches that are available to toxicologists; demonstrate application of the exposure NAM to toxicology and chemical risk assessment; clearly identify the chemical "domain of applicability" and any underrepresented chemical classes; identify obstacles to regulatory acceptance of the exposure NAM. The Workshop will conclude with a moderated panel discussion where speakers will address audience questions on how to apply the presented methods in chemical risk assessment.

**1096 New Approach Methodologies Informing Operational Air Force Mission**


Toxicologists within the Air Force are charged with providing Commanders with information necessary to make operational decisions regarding chemical exposures. In this case, the ability to predict outcomes based on environmental/individual variables is desired. In order to deliver that predictive capability, we need obtain and analyze data in a rapid, cost effective manner. Subsequently, that data can be applied to generate robust models capable of identifying chemicals that lead to biological responses and determining their mechanism of action. This process is intended to inform prioritization of specific compounds for more extensive toxicological evaluation, allowing researchers to focus on those chemical exposures with the greatest risk of contributing to mission failure/causing detriment to mission success. The Air Force employs non-animal new approach methodologies (NAMs) to provide relevant scientific foundations for human health risk assessment and broader coverage of chemicals and mixtures, with lower costs and decreased time required to obtain results. This presentation will address how the Air Force is leveraging and advancing exposure science coupled with a systems biology approach. In particular, Air Force researchers are using toxicological NAMs (QSAR, htk, PBPK, advanced in vitro models, toxin target docking) to elucidate underlying mechanisms of effects based on given sets of exposures, usually underrepresented chemical classes, from a molecular to human level perspective. Applying NAMs together with a Systems Biology approach (multiomic analyses and bioinformatic ingestion of varied data sets and types to infer combinatorial biologic effect) has allowed Air Force toxicologists to address quick-turn operational requests. This approach ultimately has great potential to determine/deliver personalized safe exposure levels and knowledge supporting individualized treatment and mitigation strategies for the extreme environments our Airman work in daily.

**1097 Filling Gaps in Exposure Data from Chemical Descriptors with Machine Learning**


One constant across exposure science is a poor data landscape for many chemicals in commerce. Fortunately, machine learning has become an increasingly common approach to fill such gaps in scientific knowledge. While machine learning is not unique to exposure science, it represents a new approach methodology (NAM) wherever it is applied. A common application of machine learning (ML) in drug-discovery and toxicology is quantitative structure activity/property relationship (QSAR/QSPR) modeling, which uses measured or reported biological activities or physiochemical properties of known chemicals to predict information data-poor chemicals, based on chemical structure descriptors. The methods used in these traditional QSAR applications are now also being used to address similar data gaps in exposure science. ML QSAR approaches include both classification and regression models; selection of appropriate specific algorithm is based on training set characteristics (e.g., size) and the specific question being addressed. This presentation will discuss recent efforts to develop robust training sets and predictive random forest classification and support vector machine regression models for chemical exposure prediction. Several chemical exposure databases are being used for estimating exposures in a high-throughput (HT) manner. Parameters that have been predicted include the functional role of a chemical in products or processes, weight fraction ranges in consumer products, probability of occurrence in environmental media, and potential pathway of human exposure (e.g., consumer, industrial, dietary). In addition, the development of ML models for toxicokinetic parameters that allow for in vitro to in vivo extrapolation of cell-based hazard data for comparison with HT exposure estimates will be covered. The presentation will conclude with a discussion of strategies for facilitating acceptance of these ML-based new approach methodologies in the regulatory arena. These strategies include adoption of transparent and open methods for communicating training sets, model development, and chemical domain of applicability and the development of frameworks that encourage iterative improvement and expansion of models as new data become available.

**1098 Prioritizing Chemicals and Research Needs Using High-Throughput Exposure Models**

J. Arnot. ARC Arnot Research and Consulting, Toronto, ON, Canada.

Thousands of chemicals require ecological and human health assessment; however, there are extensive monitoring and biomonitoring data gaps. Thus, uncertainty in exposure estimates for most chemicals is difficult to even quantify. High throughput exposure models, including New Approach Methodologies (NAMs), have been developed for addressing uncertainty in exposure assessment in various decision contexts. There is also uncertainty in the data required to parameterize and apply exposure models, e.g., use quantities and exposure scenarios, physical-chemical properties, degradation half-lives. NAMs for exposure estimation have been evaluated in case studies where monitoring and biomonitoring data exist (relatively “data rich” chemicals). NAMs for exposure estimation have been applied to thousands of chemicals to guide prioritization efforts. Screening-level uncertainty analysis provides quantitative estimates of the uncertainty in an exposure estimate. Predicted exposure estimates typically span several orders of magnitude. Fortunately, the models can be used to identify the important sources of uncertainty in human exposure and risk assessment in a systematic manner.

One approach is to compare exposure model predictions with independent measurement to obtain empirical uncertainty estimates. For example, this approach has been used in the EPA’s Systematic Empirical Evaluation of Models (SEEM) framework for hundreds of thousands of chemicals. Another approach is Monte Carlo analysis that propagates the uncertainty of chemical information (model input parameters) and/or model system parameters into exposure model calculations, e.g., air concentrations, or human intake rates. Case examples of these emerging methods and results are presented and discussed.

**1099 High-Throughput Toxicokinetics Enables Risk-Based Prioritization**

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

Chemical risk assessment requires information on hazard, exposure, and toxicokinetics (TK) relevant to the scenario, for example, consumer, ambient, or occupational exposure. Many chemicals have no or limited availability of TK data. Non-pesticidal chemicals are unlikely to have any in vivo TK data, even from animals. To fill this gap we collect key chemical-specific data in vitro. We define “high throughput toxicokinetics” (HTTK) as the combination of in vitro toxicokinetic data with generic toxicokinetic models. The primary goal of HTTK is to provide a human dose context for bioactive in vitro concentrations from high throughput screening. These methods support in vitro exposure data. As an example, the US EPA provides open source, peer-reviewed tools for HTTK in the R package “httk”. However, acceptance and use of in vitro data for hazard identification, prediction, and estimation is limited, in part, by uncertainties associated with toxicokinetics. With a generic model we do expect larger uncertainty, but also greater confidence in model implementation. We can estimate bias and uncertainty and try to correlate those with chemical-specific properties. EPA has been generating new in vitro TK data and expanding the available models to better cover key exposure routes, including dermal and inhalation. HTTK tools have been coupled to Monte Carlo simulation to allow propagation of both measurement uncertainty and biological variability into IVIVE-based chemical risk prioritizations. HTTK continues to expand the ways in which it can inform risk-based prioritization based on the relationship between in vitro bioactivities and exposures.
This presentation will discuss ongoing research activities in the EU looking into wide-scale implementation of PCLS for toxicology. Funded by EU Horizon 2020 frameworks (1), researchers have developed and optimized a new approach for standardized preparation, storage and transport of PCLS. Details will be presented on the technical advancements used in PCLS preparation, storage, and shipment. This session will showcase in the Workshop’s opening presentation by Dr. Holger Behrsing, who is part of the Institute for In Vitro Sciences Inc., Gaithersburg, MD. The Institute for In Vitro Sciences (IVS) provides veterinary toxicology testing for inhaled and other materials potentially having human pulmonary risk. Of the most relevant 3-dimensional lung models, human precision-cut lung slices (HuPCLS) offer native architecture of the respiratory parenchyma and small airways with corresponding cell types, including immune competent cells that are known to drive inflammatory and sensitization processes. Multiple laboratories are now reporting long term HuPCLS cultures (up to 4 weeks) and results that are reflective of key events in lung disease progression. However, laboratories conducting slice work are not using consistent methodology (i.e. culture method and medium), thereby complicating inter-laboratory data comparisons and HuPCLS acceptance as a standardized model. For example, a recent report has indicated insulin supplementation increases the culture medium enabling long term airway contractility studies for several weeks, but only several laboratories report its inclusion. Additional advances such as cryopreservation has highlighted HuPCLS as a pulmonary test system that should be considered for standardization. As a GLP-compliant contract research organization, IVS serves industries such as those manufacturing or evaluating pharmaceuticals, E-liquid aerosols, cleaning and personal care products, environmental chemicals and particulates, and others that may require regulatory oversight for their materials. A harmonization approach that could be applied in the absence of sufficient hazard characterization and data. The delivery of vision and the tools are aspired to make future BEA developments consequential, scientifically meaningful and proportionate/commensurate with the spirit and goals of REACH. In addition, a newly proposed screening method for identifying and attributing chemicals to co-exposure groups of importance as a first step towards a “real-life” mixture exposure and cumulative health risk assessment. Generally, co-exposures are anticipated either because of co-exposure of substances within a formulated product, anticipated co-use or co-exposure in the environment. This approach employs high-throughput human exposure modeling with minimal data to establish scenario-specific groups of chemicals with likeliest/highest potential for (external) co-exposure based on the identified commonalities/differences in chemical exposure patterns. The resulted groupings are compared with co-exposure concentrations reported from in vitro data, and conclusions are drawn as the direct measure of uncovered biologically relevant co-exposure in humans. When combined with relevant biological and toxicological information the external co-exposure groups yield the risk-based categories of chemicals relevant for high-tier cumulative risk assessment.

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Studies of airway responsiveness have employed PCLS for over 30 years, mainly to elucidate the mechanisms of asthma exacerbation. The presentation will highlight the applicability of this model for the study of several types of environmental exposures of the human lung including formaldehyde, salicylic acid, toluene diisocyanate, and 1-chloro-2,4-dinitrobenzene. The physiologic and cellular signaling endpoints in PCLS associated with airway responsiveness caused by these substrates and others will be summarized. The mechanisms underlying airway responsiveness from cell-cell interactions will be demonstrated through studies of functional mast cells in PCLS. Special focus will be given to techniques utilized in assessment of airway contractility/relaxation in non-diseased human tissue. Investigations of airway responsiveness using PCLS will be placed in the context of inflammatory lung diseases, as well as in examination of airway hyperreactivity after exposure to environmental triggers and exacerbators of underlying airways diseases.

**1104 Assessment of Airway Responsiveness in Human Precision-Cut Lung Slices: New Applications of an Established Method**


**1105 Early Events of Pathogenesis of Respiratory Diseases Induced by Agents in Human Lung Tissue**

K. Sewald. Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany. Sponsor: J. Herbert

According to the 3Rs principle and for improved prediction of chemical toxicity and adverse drug reactions models other than animals are used to assess chemical safety. Human lung tissue is complex, contains many different structural cell types and resident immune cells. As the lung is immune competent, changes to the release of pro-inflammatory cytokines and chemokines are often associated with injury, inflammation, fibrosis and even agent induced immune suppression. This presentation will show how the use of viable human tissue improves experimental design for acute toxicity testing. The acquisition of human tissues remains a significant challenge. We receive human lung tissue from two hospitals in Hannover, Germany. High tissue quality is achieved through a standardized approach to tissue collection with the application of appropriate quality controls. Lung tissue is exposed either submerged or at air-liquid interface to chemicals, proteins or complex mixtures such as cigarette smoke. The combination of different techniques, the use of end-stage diseased and healthy tissue provides a great opportunity for identifying chemical-changed biomarkers and pathways. The presentation will give an overview of what it means to work with human tissue to drive discovery and safety of drugs and chemicals. Examples will include the combination of toxicity testing and prediction-cut lung slices for assessment of drugs such as nitrofurantoin, chemicals such as isocyanates and complex mixtures such as cigarette smoke. The examples will be embedded into relevant adverse outcome pathways linking the molecular level to an adverse outcome (respiratory injury, inflammation, sensitization, pulmonary fibrosis). Immune suppression after treatment due to immune-related adverse events, may even lead to an increased risk for infection with e.g. influenza. Human lung tissue increases the predictive validity of disease models by considering interspecies differences for comparative pharmacology and toxicology, and most importantly by providing a reference point associated and correlated with clinical symptoms. Although many scientists know of the undoubted potential of PCLS to contribute to drug development for early-stages of this disease. Species differences in sensitivity to therapy will be exemplified in PCLS exposed to the only FDA-approved idiopathic pulmonary fibrosis drugs, Pirfenidone and Nintedanib. The role of PCLS within our drive for precision medicine and toxicology is growing to efficiently study the potency of therapeutics and toxicants in healthy and diseased human donor tissue.

**1106 From Acute Exacerbations to Chronic Pathologic Processes: Translational Benefits of PCLS For Studies of Chronic Lung Disease**

M. Koenigshoff. University of Pittsburgh, Pittsburgh, PA. Sponsor: J. Herbert

This presentation is focused on the extended use of PCLS to study chronic lung diseases and to provide critical translational information between animal disease models and human risk assessment. To highlight PCLS suitability for human chronic disease modeling beyond the use of scarce end-stage tissue, methodologies to induce the on-set of cellular senescence and pulmonary fibrosis in healthy donor tissue will be introduced. Mechanistic studies of cellular senescence in the alveolar region of PCLS caused by altered WNT/β-catenin signaling will be shared in the context of lung aging and idiopathic pulmonary fibrosis. A technique to generate qualities of early-stage fibrosis in PCLS from healthy human tissue will be presented as an example of the potential of PCLS to contribute to drug development for early-stages of this disease. Species differences in sensitivity to therapy will be exemplified in PCLS exposed to the only FDA-approved idiopathic pulmonary fibrosis drugs, Pirfenidone and Nintedanib. The role of PCLS within our drive for precision medicine and toxicology is growing to efficiently study the potency of therapeutics and toxicants in healthy and diseased human donor tissue.

**1107 Evolving Technologies for Determination of Biotherapeutic Specificity**

T. MacLachlan. Novartis AG, Holliston, MA.

Monoclonal antibody therapeutics evolved from low molecular weight drugs with the promise of substantially higher specificity than low molecular weight drugs, and thus with much lower potential for toxicity. Since safety of monoclonal antibodies is directly linked to the specificity of their binding to therapeutic targets, developers of monoclonal antibody therapeutics have evaluated specificity of their products using the “Tissue Cross Reactivity” assay, based on an immunohistochemical method where the drug candidate is panned across a large number of human tissues. Recently, several platforms for screening for potential cross reactivity have become available, from protein and cellular arrays to flow cytometry-based methods, and their utility as possible replacements of immunohistochemistry method has been considered. This session will address the growing options that sponsors have to evaluate specificity of monoclonal antibody pharmaceuticals in the preclinical safety package.

**1108 “TCR 2.0”: Reviewing Experience with Methods to Detect Off-Target Binding of Monoclonal Antibodies**

J. Cavagnaro. Access Bio L.C., Boyce, VA.

Monoclonal antibodies (mAbs) and derivatives thereof have become a mainstay pharmaceutical modality. A primary benefit of these proteins is their highly targeted nature which can reduce the incidence of side effects in patients. It is critical however to ascertain the specificity of antibodies prior to human clinical trials, and several country and worldwide guidances direct sponsors to evaluate this. For the last two decades, the primary technique for determining specificity has been the immunohistochemistry (IHC) based “Tissue Cross Reactivity” assay (TCR), where the candidate antibody is panned across 32 tissues to look for unexpected staining. In the last few years however, other array-based platforms have emerged that allow for screening the majority of the human membrane proteome, indicating a viable alternative and/or addition to the IHC methods. Additionally, a “Q&A” to the ICH S9 guidance in 2018 indicated that TCR assays are not required in most situations for oncology biotherapeutics. The preclinical committee of the Biotechnology Innovation Organization (BIO), “Biosafe”, has conducted a survey of 26 BIO member companies to understand current sponsor experience with the IHC and array techniques for determining antibody specificity. In the last ten years, more than 650 IHC TCR assays have been conducted largely on full length mAbs with varying impacts on programs. Protein/cell arrays have been utilized by a third of the companies surveyed and are gaining familiarity and comfort with the platform – initial experience with recent versions of these arrays has been largely positive and are integrating new modalities such as scFvs from CAR-T therapies. ICH S6R1 guidance offers sponsors the option to use alternatives to IHC to determine antibody specificity – while most sponsors are not prepared to eliminate the IHC method, growing experience with these alternatives may allow them to confidently choose one with or without IHC in the future. Details on sponsor responses and experience will be shared in this session.

**1109 Immunohistochemistry-Based Tissue Cross-Reactivity Studies: History and Current Perspective**

B. Beutow. Pfizer Inc., San Diego, CA. Sponsor: T. MacLachlan

Immunohistochemistry (IHC) has traditionally been used as the primary method to assess tissue cross-reactivity (TCR) for monoclonal antibody and antibody-based therapeutic candidates. More recently, TCR studies have been used to determine the potential cross reactivity of more novel therapies such as T cell-engaging bispecifics and CAR-T cells, which can have potent and potentially long-lasting effects. TCR studies have been recommended since the early 1980’s by various regulatory guidance documents which have been modified over time to the most recent Points to Consider (FDA, 1997) and ICH S6 (R1) (2011) documents. TCR studies incubate the candidate antibody/
molecule with a wide range of tissues and detect binding of the candidate antibody using a variety of IHC techniques. In this regard, TCR studies using IHC have the unique ability to test for cross-reactivity across many tissues from both humans and animals. An evaluation can be made about which cell type is involved, as well as whether the binding appears to be on the cell membrane vs cytoplasm. While TCR studies sometimes provide key information about potentially cross-reactive targets, they are also prone to false positive results (as assessed by the presence of findings in “positive” tissues in in vivo toxicity studies), which can have negative impact on drug development. This is in part because the candidate antibodies/molecules are selected for their ability to have a desired therapeutic effect in vivo, and not because they are a good IHC reagents. Several case examples will be presented to highlight the variable results obtained with TCR studies.

### 1110 Protein- and Cell-Based Arrays to Assess Specificity of Biotherapeutics

**A. Vicart. Novartis AG, Basel, Switzerland. Sponsor: T. MacLachlan**

Off-target binding of biotherapeutics to either cell surface or secreted proteins can lead to attrition in drug development. With new modalities such as antibody drug conjugates (ADCs), bispecific antibodies targeting T-cells and CAR-T therapies, a lack of specificity could lead to severe side effects in patients. In the last few years, protein and cell array-based platforms have emerged that allow for screening against an extensive range of human proteins. We have and are continuing to test various platforms including non-contact inkjet printed protein arrays and cellular arrays in slide, plate and flow cytometry formats. While some platforms are still under evaluation, we have implemented a revised strategy to profile the specificity of biotherapeutics including, a) profiling cross reactivity potential on a cellular array prior to formal toxicity studies, b) immunohistochemistry on a panel of tissues using an IHC-optimized tool antibody against the intended target and c) including screening results in the IND package together with the formal IHC-based Tissue-Cross-Reactivity (TCR) for non-ondcology indications or as a substitute to TCR for oncology or when an immunohistochemistry method was not able to be developed. Initial experience with this strategy with more than 90 molecules profiled has been largely positive. Undisclosed on-targets were identified in more than 90% of screens and 2/3 of identified off-targets were confirmed by alternative binding and/or functional assays. Several examples will be shared including its use for IND submissions, regulatory acceptance of these approaches, as well as how it helped selecting the best candidate to terminate non-selective lead biotherapeutics with financial and animal use reduction impacts.

### 1111 The Methodological Road toward Single Cell High-Throughput Transcriptomics (scHTTr)

**B. Chorley. US EPA, Research Triangle Park, NC.**

Measuring transcriptomic events at a single cell level has revolutionized our understanding of cellular biology and response to the environment. In a high-throughput transcriptomics (HTTR) screening setting, single cell measurements reduce background noise associated with bulk RNA-sequencing technologies, providing more accurate assessment of adverse cellular response. Single cell assessments also increase the fidelity of transcriptomic alterations in organotypic screening models or complex cellular makeups of in vivo samples of animal test models for later tiered chemical testing. Unfortunately, the hundreds of thousands of samples performed in a high-throughput screening setting are not conducive for most established single cell methods due to the high costs and bioinformatically intensive environment associated with this type of data. However, recently introduced methodology and Informatic approaches are providing a clear path forward for single cell high-throughput transcriptomics (scHTTr). In this Workshop, the first two presenters will describe developing technologies that will reduce the read depth, increase the throughput, and significantly decrease the cost of single cell transcriptomic changes in toxicological screening batteries conducive for toxicological and drug-screening applications (Single Cell TempOSeq and sci-Plex). The final speaker will present a case study using a scHTTr method to assess the effects of Polycyclic Aromatic Compounds (PACs) mixtures on resident immune cells and progenitors in the adult mouse bone marrow as part of a comprehensive hazard assessment. With these recent advancements, scHTTr is now at the point of implementation into toxicological assessments to increase the measured resolution of transcriptomic responses and better assess the potential adverse health impacts of toxicant and drug exposures. This abstract does not necessarily reflect US EPA policy. Mention of trade names is not an endorsement or recommendation for use.

### 1112 Reducing Read Depth and Increasing Throughput and Sensitivity for Single Cell Transcriptomic Analysis Using Single Cell TempO-Seq


High throughput transcriptomic analysis of single cells permits identification of rare cell types within large populations and their responses to environmental or genetic perturbations. Single cell responses to exposure are heterogeneous, which can influence adverse effects in tissue and the organism as a whole. Therefore, transcriptional profiling of individual cells following chemical perturbations is highly informative to toxicological studies. With current single cell approaches such as RNA-seq, low sensitivity hinders measurement of moderate-to-low abundance transcripts that play significant biological roles. RNA-Seq also generates non-informative reads that take up sequencing space and increase costs. In this presentation, we describe the Single Cell TempO-Seq® gene expression assay. The targeted nature of TempO-Seq permits significantly less read depth while sequencing gene expression profiles correlate to RNA-Seq but require five times less sequencing depth. Indeed, we have observed that Single Cell TempO-Seq® is highly sensitive to low expressed genes, measuring 10,000 genes per cell compared to typical sensitivity of traditional single cell methods of 2,000 to 4,000 high/moderate expressed genes. A Single cell TempO-Seq data set down-sampled to an equivalent read depth of a library produced using the 10x Genomics Chromium system produced greater than three times the number of genes detected per single cell. Because of the probe-based nature of the assay, this method does not require poly-adenylated or intact RNA and can therefore measure non-coding RNA species (splice variants, fusion genes, single base variants, long non-coding RNA, etc). The ability to use fixed cells permits banking multiple time points or cell preparations for concurrent input into the assay, and one can easily multiplex many samples made up of individual cells. Finally, the assay works on intracellular or extracellular antibody stained cells, permitting the assessment of a whole-cell view of tissues and organs. We have implemented a revised strategy to profile the specificity of biotherapeutics. We applied sci-Plex to comprehensively profile the transcriptional response of 3 cancer cell lines to 188 compounds targeting diverse cellular properties at various doses. In total, we profiled close to 5,000 unique conditions across 649,340 single-cell transcriptomes all within one experiment. Our analysis identified heterogeneous responses of cells to specific compounds as well as commonalities in the transcriptional response of cells treated with compounds of similar mechanisms of action. Additionally, our observations of the response of cells exposed to one of several HDAC inhibitors supports the view that chromatin acts as an important reservoir of active cells which can be leveraged to characterize the single-cell resolution of our approach to define the drug-induced molecular response of natively-heterogenous cancer models. These efforts will result in an actionable description of the dynamic changes that underlie therapeutic response in cancer.

### 1113 Defining Drug-Induced Molecular Landscapes with Multiplex Single Cell Genomics

**J. McFaline. Columbia University, New York, NY. Sponsor: B. Chorley**

The exposure of cancer cells to therapy induces complex changes that can positively or negatively alter clinical outcome. Defining the molecular landscapes that accompany drug exposure has the potential to identify new and effective combination therapies. To arrive at these comprehensive maps of drug-induced molecular changes, we need a method capable of profiling the response to treatment of many genetic backgrounds, ideally at single-cell resolution to account for heterogeneity in response, at the level of sub-clones and/or cell states. We recently developed “sci-Plex,” a method that combines sample multiplexing via nuclear “hashing” and the high-throughput of single-cell combinatorial indexing RNA-seq (sci-RNA-seq) to create a platform for multiplex single-cell chemical transcriptomics. We applied sci-Plex to comprehensively profile the transcriptional response of 3 cancer cell lines to 188 compounds targeting diverse cellular properties at various doses. In total, we profiled close to 5,000 unique conditions across 649,340 single-cell transcriptomes all within one experiment. Our analysis identified heterogeneous responses of cells to specific compounds as well as commonalities in the transcriptional response of cells treated with compounds of similar mechanisms of action. Additionally, our observations of the response of cells exposed to one of several HDAC inhibitors supports the view that chromatin acts as an important reservoir of active cells which can be leveraged to define the single-cell resolution of our approach to define the drug-induced molecular response of natively-heterogenous cancer models. These efforts will result in an actionable description of the dynamic changes that underlie therapeutic response in cancer.

### 1114 Exposure to Polycyclic Aromatic Compound Mixtures Impacts Resident Immune Cell Progenitors in the Bone Marrow of Adult Mice

**O. Lozoya. NIEHS, Research Triangle Park, NC. Sponsor: B. Chorley**

Polycyclic Aromatic Compounds (PACs) are ubiquitous environmental contaminants that result from incomplete combustion of organic material. These contaminants are always occurring mixtures, and even a single PAC mixture can be a mediator of the capacity of studies on PAC-induced toxicity in laboratory or controlled environment to effect actionable public health and environmental policy.
One common means of exposure to PAC mixtures is through tobacco smoke which, as we recently reported, leads to an ‘aging’ phenotype in circulating immune cells. In this context, we developed a cross-sectional NIEHS/NTP project to characterize how exposure to PAC mixtures at different stoichiometries affects resident immune cells and progenitors in the adult mouse bone marrow, and infer potential long-term consequences of such insults for the immune system, by single-cell transcriptomics in combination with endogenous spike-in libraries for bulk-level epigenetic profiling using an automated high-throughput scRNA-seq system implemented in-house. Our experimental design comprises 28 days of oral exposure of female B6C3F1/N mice (8-12 weeks old at start of dosing) to equipotent mixtures of 13 distinct PACs. The constitution of equipotent PAC mixtures is based on the effective dose of each PAC that caused 10% suppression (Eqp ED10) of the humoral immune response to sheep red blood cells (SRBC) when tested individually. These data will be used to determine the potential of Eqp ED10 PAC mixtures to induce immunotoxicity as part of a comprehensive hazard assessment for this model mixture. By applying leading-edge tools to address high priority environmental health issues which, like PACs, present a public health concern in multiple different exposure scenarios, our work embodies a reference framework on how to coalesce emerging technologies, such as high-throughput single-cell transcriptomics, into ongoing toxicological studies and enhance their relevance to health policy making.

1115 Challenges and New Approaches in Characterizing Toxicity within the Military

T. D. Vincent, Department of Veterans Affairs, Washington, DC.

As more than 20 million US citizens either have served or are currently serving in the military, the Department of Defense and Veterans Affairs are tasked with identifying the potential threats that these individuals may encounter and ensuring their safety while in service and quality of life after separation. Military service involves a vast and unique set of toxicological considerations when compared with most occupational settings, which can vary for each individual, depending upon the mission. While some of these hazards, such as many organic solvents, are not unique to military service, and parts of their toxicity profiles have been established, other threats to human health, such as materials developed to achieve certain mission-specific outcomes, have emerged that are not well understood. As these threats evolve, the need to characterize their associated toxicities requires novel, progressive approaches that transcend classic methodologies. As representative examples of the methods being used to understand the unique health consequences of military service, the speakers in this session will discuss: (1) surveillance of veterans who endured blast injuries during deployment; (2) designing a framework to elucidate intergenerational effects of military exposures; (3) evaluating the long-term health implications of occupational jet fuel exposure; and (4) employing tools to characterize chemical threats and provide guidance for treatment. These topics will highlight some hazards and exposure pathways specific to military personnel and the challenges involved in maintaining their health during and after their service.

1116 Biomarkers of Exposure and Early Effect in the Medical Surveillance of War-Injured Veterans with Retained Metal Fragments

J. M. Gaitens, and M. A. McDermid, University of Maryland School of Medicine, Baltimore, MD.

Retained metal fragments resulting from combat-related contact with a blast or explosion pose a unique long-term health threat to war-injured Veterans. Evidence has shown that oxidation of these retained fragments in situ can result in ongoing systemic metal exposure potentially impacting target organs far from the site of injury. Two distinct metal-exposed populations of war-injured veterans, one exposed to depleted uranium during the 1st Gulf War and the other injured primarily from improvised explosive devices during the more recent conflicts in Iraq and Afghanistan, have been followed by the Department of Veterans Affairs using medical surveillance protocols to identify potential health effects. These protocols include the use of urine biomonitoring to characterize exposure and measures of organ system functional outcomes, as well as novel early effect biomarkers to survey for target organ insult. We will present current surveillance findings for both at-risk populations.

1117 A Retrospective Investigation of the Long-term Health Implications of Occupational Jet Fuel Exposure in the Air Force

T. D. Vincent1, G. Wolf2, J. Esposito2, and W. J. Culpepper3, 1US Department of Veterans Affairs, Washington, DC; 2US Air Force School of Aerospace Medicine, Wright-Patterson AFB, OH; and 3Department of Veterans Affairs, Washington, DC.

Exposure to jet fuels is one of the most commonly experienced occupational hazards in the military across all branches of service. Although the acute effects of these exposures have been well-documented, less is known about the long-term implications of these occupational exposures for health. The Department of Veterans Affairs and the US Air Force School of Aerospace Medicine conducted a study to determine associations between occupational exposure to jet fuels and adverse health outcomes. Occupation codes were categorized by potential level of exposure to jet fuels, and time-in-job and deployment history were among the factors used to characterize the overall exposure experience of Air Force personnel. Our findings suggest that occupational exposure to jet fuels is associated with chronic health outcomes that occur in various organ systems, including the nervous and respiratory systems, as well as renal and dermal conditions. These associations appear to be a function of intensity/frequency of exposure, as well as duration of occupational duties. Future studies are needed to further investigate these relationships and better inform policy on the use of personal protective equipment and the need for routine monitoring of health conditions associated with occupational exposure to jet fuels.

1118 Generational Effects of Military Exposures to Chemical Toxics of Interest

M. A. Williams1, V. Davey2, and K. Block2, 1US Army Public Health Center, Aberdeen Proving Ground, MD; and 2US Department of Veterans Affairs, Washington, DC.

Over the past 20 years or more, Military Service personnel have experienced multiple deployments to major theaters of operation, including the 1990-1991 Gulf War and prolonged conflicts in both Iraq and Afghanistan following the 9/11 terrorist attacks on the United States. It is now recognized that Veterans of those conflicts experienced a variety of exposures to toxicants of unknown concentrations and combinations, with unclear health impacts. These exposures include, but are not limited to, vaccines, chemical nerve agents, airborne hazards, radiological hazards, and possible exposure to social and psychological stressors. The concerns of Veterans regarding impacts of deployment-associated exposures to their reproductive and non-reproductive health have expanded to generational health impacts that might be seen in their descendants. The environmental and exposure influences on fetal- and childhood-development is emerging as a critical area of investigation. The complexities to identify and unravel the mechanisms underlying toxicant- and stress-induced functional changes in offspring remain challenging. The National Academies of Sciences, Engineering and Medicine (NASEM), reported in Gulf War and Health, Vol.11, Generational Effects of Serving in the Gulf War that 27 chemicals of interest associated with Military service could have reproductive, birth or developmental health effects, but did not find sufficient evidence that these specific exposures were linked to major birth defects in the children of Gulf War and OIF/OEF military Service member populations. A comprehensive Health Monitoring and Research Program (HMRP) was suggested to longitudinally monitor exposures in, and health of, Military personnel and their descendants. This approach encompasses epidemiological and molecular methods through the collection of data and biological specimens. To determine the feasibility and scalability of an HMRP, a panel of subject matter experts in the areas of epidemiology, toxicology, molecular biology, pediatrics, and reproductive and developmental biology were convened. Academic and Federal outside experts were invited as needed for consultation. This presentation will review and invite discussion on our progress in developing a toolbox of resources and phased approaches for an HMRP to provide critically needed answers to Veterans and policymakers.

1119 Predictive Toxicology in Preparation for the Unknown Threat

K. Glover, US Army Combat Capabilities Development Command Chemical Biological Center, Aberdeen Proving Ground, MD.

Traditional approaches relying solely on animal models have become obsolete. In order for our warfighters to maintain a unilateral advantage in the presence of a potential unknown chemical threat, the US Army CCDC Chemical Biological Center is building, integrating, validation and imple-
menting next-generation tools to support toxicological characterization of hazardous chemicals. These tools comprise a predictive toxicology toolbox combining machine learning (QsarAR, three-dimensional ligand docking), physiologically based pharmacokinetic modeling, high-throughput platforms (engineered cell screens), high-content platforms (GPCRome screening, multi-omics), human microphysiological test systems (organ-on-a-chip) and hypothesis-driven in vivo test schemes. This talk will describe how these tools have been utilized to date for traditional nerve agents, chemical weapon precursors and/or pharmaceutically based agents as we move toward building a systematic framework for characterizing the emerging unknown chemical threat of the future.

**1120 Development of Medical Countermeasures for Chemical Warfare Nerve Agents: A Multimodal Approach**


Intentional exposure to chemical warfare nerve agents poses a real-world risk to Warfighters. This is highlighted by recent incidents in Syria and the United Kingdom. To mitigate this for deployed personnel, it is imperative not only to understand the mechanism of toxicity of chemical warfare nerve agents but also to develop effective medical countermeasures and clinical practice guidelines for treating exposed individuals. New approaches in the field of nerve agent medical countermeasures encompass the development of humanized animal models to more accurately represent nerve agent toxicity, design of novel centrally active acetylcholinesterase reactivators that cross the blood-brain barrier, and new evidence-based studies to provide data for rational selection of FDA-approved therapeutics to treat nerve agent poisoning in a military operational context, chemical exposures and trauma combine to produce injuries that are challenging to treat. The development of appropriate combined injury animal models and treatment strategies addresses this concern. Thus a strategic multimodal approach addresses the development of effective treatments to protect Warfighters from the toxic effects of chemical warfare nerve agents.

**1121 Identifying and Communicating Adverse Neurological Outcomes from Parental Cannabis Use**

K. Ryan. NIEHS/NTP, Research Triangle Park, NC.

The expansion of legal cannabis in the United States brings with it increased use and consequently increased risk of adverse effects. Cannabis use by pregnant women in the United States has also increased as much as 62% (2002-2014) with an overall prevalence of use between 3%-16%. One emerging area of concern has risen from epidemiological evidence highlighting a variety of neurological impairments in children associated with maternal cannabis use during pregnancy. In parallel, experimental animal studies have demonstrated that paternal THC exposure causes neurodevelopmental and neurobehavioural impairments in offspring. Additional data from clinical and experimental animal studies have revealed that abnormal epigenetic imprinting on sperm is associated with parental cannabis use. These findings point to important risks of cannabis use on reproduction and could be a mechanism for adverse neurodevelopmental deficits in the next generation. However, our understanding is not nearly complete, which could contribute to some confusion among consumers or a lack of concern related to cannabis use prior to conception or during pregnancy. In this session, the first speaker will begin by describing the use of cannabis for pain and nausea by women during pregnancy and while breastfeeding. Some misconceptions regarding cannabis use and risk among consumers also will be presented. As a result of increased use, longitudinal studies are demonstrating a link between fetal cannabis exposure and decreased growth, cognitive impairment, and behavior deficits in children. The second speaker will provide evidence in rodents corresponding to clinical findings that allows for in-depth assessments of neurobehavior and associated molecular phenotypes. Results from human and animal studies also will be discussed in the context of generating education strategies or interventions to improve the mental health outcomes of children with early in utero cannabis exposure. In addition to maternal exposure, the third speaker in this session will highlight new evidence in preclinical rodent models showing that paternal exposure to cannabinoids prior to conception causes neurobehavioral impairment in the offspring, possibly through changes in DNA methylation. Paternal exposure impacts on offspring neurodevelopment is a largely understudied area. The next speaker strengthens the weight of evidence by linking parental cannabis exposure and adverse effects on neurodevelopment in a third species (i.e., zebrafish). Use of this complementary model system allows for the assessment of cannabis as a complex mixture on development, behavior, and reproduction across multiple generations, which is often a resource-intensive task in rodent models or human studies. The final speaker in this session provides insight from the public health perspective, focusing on the real-world application and utilization of preclinical and clinical research critical to the development of public health recommendations and risk communication. The session will feature an informal deliberation among panel speakers and the audience to (1) review the current weight of evidence for neurological deficits in children as a result of parental cannabis use during pregnancy, (2) propose strategies for research data gaps, and (3) discuss communication strategies to highlight risks for consumers. This session brings together experts across clinical and preclinical research settings, including several non-SOT members, to provide a platform to discuss and further our understanding of the impact of cannabis use on subsequent generations.

**1122 Is Prenatal Marijuana Use Harmful? A Clinician’s Perspective**

T. Metz. University of Utah, Salt Lake City, UT. Sponsor: K. Ryan

As legalization of marijuana increases across the United States, so does the prevalence and perceived safety of marijuana use in pregnancy. Some women cite reasons for marijuana use in pregnancy and while breastfeeding such as nausea, pain and anxiety. A National Academies of Sciences report on the health effects of cannabis concluded that marijuana use is associated with poor fetal growth but that evidence for the association between marijuana use and other perinatal outcomes is inconclusive. Limitations of the existing literature which preclude firm conclusions as to the effects of prenatal marijuana use include a high reliance on self-report for ascertainment of use and incomplete adjustment for confounding factors such as tobacco use. To avoid biased or inaccurate information gathering, biological sampling for marijuana metabolites will be a useful adjunct for future clinical research. There are three longitudinal studies examining the relationship between prenatal marijuana exposure and childhood neurodevelopment. While early childhood neurodevelopmental outcomes are similar, investigation later in life demonstrates decreased attention, verbal reasoning and cognitive function. With the high prevalence of marijuana use among reproductive age women, it is critical for healthcare providers to query women regarding use and provide information regarding the potential harms. Women should also be asked about reasons for use, as a strategy to develop alternative care that can often be provided. From a public health standpoint, the importance of clinicians partnering with representatives of the cannabis industry to ensure appropriate messaging to pregnant and breastfeeding women will be emphasized. Given the available evidence, women should be advised to refrain from marijuana use during pregnancy and while breastfeeding.

**1123 Neurodevelopmental Outcomes of Early-Life Cannabis Exposure**

A. Bara. Icahn School of Medicine at Mount Sinai, New York, NY. Sponsor: K. Ryan

The expanding legalization of recreational and medical cannabis as well as the changing attitudes regarding the harm of cannabis has contributed to a significant increase in women using cannabis while pregnant. Additionally, there is increased prevalence of cannabis consumption among breastfeeding women and of children with second-hand cannabis exposure. Delta-9-tetrahydrocannabinol (THC), the psychoactive component of cannabis, readily crosses the placenta barrier and is also transferred through breast milk which raises significant concern about the impact of the early life cannabis exposure during these critical periods of development. The goal of our project is to examine the trajectory of behavioral and neurobiological disturbances associated with early life cannabis/THC exposure. Preclinical rodent models of disrupted human fetal brain development and long-term human investigations were investigated in relation to early life cannabis/THC exposure. Biochemical, molecular, epigenetic and behavioral studies were carried out at different developmental periods. Findings from our human studies and animal models (including a vaping model), in combination with results...
from the field, emphasize neurobehavioral, socio-emotional, physiological, molecular, and epigenetic consequences of developmental cannabinoid exposure that extends from the neonatal period through adolescence and into adulthood. For example, daily maternal exposure to THC (resulting in 10ng/mL plasma concentration; ~1 Joint/day) during pregnancy alters the rat placenta transcriptome as well as molecular mechanisms related to synaptic plasticity as well as the post-embryonic linked to disturbances of histone methylation epigenetic markers and depression-like behavior. The stress system appears to be a particularly critical biological substrate affected by the developmental effects of cannabis/THC. The accumulating data emphasizes long-term impact on specific neural systems and phenotypes predictive of psychiatric disorders and vulnerability that further provide sufficient evidence to help guide interventions and education strategies to improve the mental health outcomes of children and adults.

**1124 Paternal Pre-conception THC and Cannabis Exposure of Rats Causes Long-Lasting Neurobehavioral Dysfunction in Their Offspring**

E. Levin. Duke University, Durham, NC.

It has been widely documented that maternal exposure during gestation to a variety of toxicants has been widely shown to cause neurobehavioral toxicity in the next generation. In contrast the impacts of pre-conception paternal toxicant exposure on offspring neurobehavioral function have been much less studied. We have found that preconception exposure to delta-9-tetrahydrocannabinol (THC) in male rats significantly alters sperm DNA methylation and that cannabis smoking in human males is significantly associated with altered sperm DNA methylation in similar pathways (Murphy et al., Epigenetics, 13:1208-1221, 2018). In the current studies, we investigated the observed preconception THC on offspring neurobehavioral development. We exposed adult male rats (0 or 2 mg/kg/day for 12 days or 0, 2 or 4 mg/kg/day for 28 days) to the neurobehavioral development of their offspring. These dose regimens of paternal THC exposure were not found to significantly impact the clinical health of the fathers or offspring. However, they did cause a long-lasting significant effects on locomotor activity, emotional function, and cognition in the offspring relative to controls when they were tested in adolescence and adulthood. The shorter paternal THC exposure caused a significant increase in habituation of locomotor activity and attentional impairment in the adult offspring of the males exposed to THC prior to mating. The longer paternal THC exposure caused significant locomotor hyperactivity in the adolescent offspring, significantly quicker habituation in novel object exploration and significantly delayed acquisition of the radial-arm maze in adult offspring. Paternal exposure to the complex mixture of cannabis extract containing THC before conception was found to cause significant learning impairments in female offspring and increased offspring risk-taking behavior regardless of sex. These studies demonstrate that preconception paternal THC exposure even at a modest dose can cause deleterious neurobehavioral effects in their offspring, including cognitive impairment. Additional research is needed to determine the degree to which this type of neurotoxic risk in offspring is seen in humans, to investigate the mechanisms underlying these effects and to develop therapeutic treatments to ameliorate these long-term adverse behavioral consequences of paternal pre-conception THC exposure.

**1125 Delta 9-Tetrahydrocannabinol Developmental Exposure Affects Zebrafish Long-Term Neurobehavior and Aging Phenotypes**

K. Willett. University of Mississippi, University, MS.

Availability of cannabinoids such as delta 9-tetrahydrocannabinol (THC) is increasing. Therefore, understanding potential adverse outcomes following exposure to cannabinoids during critical early developmental periods is important. Zebrafish (Danio rerio) as a research organism provide a complementary and relatively high throughput model to assess the dose-dependence of toxicity following THC exposure. After exposure to 0.08 to 2 µM THC through the larval stage, developmental deformities, behavioral analysis, and RNASeq analysis of the transcriptome were used to identify potential target organ and toxicity pathways at the neurodevelopmental level. Additionally, developmentally exposed F0 and subsequent non-exposed F1 were assessed at 30 months of age for reproductive fitness, locomotive and anxiolytic behaviors, and aging phenotypes including liver expression of genes involved in proliferation (p33), cell cycle arrest (p16, p21), and immune markers (tnfa, Il-6, Il-1b). THC's effects were biphasic and sex-specific for many of the endpoints observed including survival, weight and length dysphasia, and the expression of tnfa and Il-18. High concentration F0 exposure of THC resulted in reproductive toxicity and significantly reduced survival and reproduction in F1 progeny as adults. The lower concentrations of THC reduced the expression of genes related to senescence and inflammation and increased survival. The biphasic nature of the THC dose-response following early developmental exposure highlights the importance of considering dose-dependence in long-term and multigenerational studies of THC effects. Despite developmental exposure to low concentrations of THC increasing lifespan, none of the THC concentrations measured were found to significantly reduce in locomotor behavior indicating a lack of improved healthspan.

**1126 Communicating Public Health Concerns for Marijuana Use among Pregnant and Breastfeeding Women**

E. Contreras. Colorado Department of Public Health and Environment, Denver, CO. Sponsor: K. Ryan

The acute and long-term consequences of marijuana use during pregnancy are largely unknown. This is highly concerning to public health in Colorado as marijuana use data among pregnant and breastfeeding women are trending upward. However, longitudinal studies and more data are needed to better understand the risks and communicate the implications of marijuana use on neurodevelopmental disorders in offspring. The Colorado Department of Public Health and Environment’s Marijuana Health Monitoring and Research Program (MHRP) has an obligation to monitor changes in drug use patterns and the emerging science relevant to the health effects associated with marijuana use. MHRP created an expert panel of health professionals in 2014 to monitor the data and peer-reviewed literature and used that information to develop protective public health statements and recommendations in order to educate and protect the health of Coloradans. Our systematic approach to this work will be discussed as well as the resulting 26 evidence-based statements on marijuana use during pregnancy and breastfeeding. These statements, of varying levels of evidence, include; reduce cognitive function, lower IQ, attention problems in exposed offspring, birth defects, motor deficits, and mental health issues. High quality, epidemiological studies in this population are desperately needed to further evaluate the association(s) of these outcomes with maternal use and better quantify the exposure. MHRP funded a study on THC concentration in breastmilk; results will be discussed. Funding of research in vulnerable population like this should be encouraged, especially in legalized states. Priority should be given to research within the marijuana-using pregnant and breastfeeding population; specific topics will be discussed. Until the effects of marijuana use on exposed offspring are described in the literature and supported by data, we will not know the true impacts of marijuana legalization on the health of our future populations.

**1127 It is Not Just Air: Exposure to Indoor Air Pollution, Diagnostic Tools, and Evaluation of Health Effects**

E. Mutlu. NIEHS/NTP, Research Triangle Park, NC.

Indoor air quality continues to be a concern for developed and developing nations because of the increasing amount of time people spend indoors (> 90%). Indoor air, whether in working environments or households, can contain both gaseous and particulate pollutants, including toxic chemicals and microorganisms. Household air pollution has been reported to cause ~ 4 million premature deaths annually. Overall, indoor air pollution is the leading cause of adverse health outcomes such as lung cancer, as well as cardiovascular and pulmonary diseases in children and adults. In the United States, approximately half a million households are exposed to indoor air pollutants, with the majority of the exposure coming from burning of solid fuels like wood.

Wood smoke contains a complex mixture of compounds such as polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), and particulate matter (PM). Exposure to fine PM (PM, 2.5) has been studied extensively due to its potential to cause more hazardous outcomes especially in higher health risk groups, including pregnant women, children, elderly, and people with underlying health issues. The use of personal exposure monitors to improve the understanding of household air pollution and to help inform strategies for reducing the exposures has been utilized in several studies. The vast variety of indoor air pollutants, including VOCs, PAHs, PM, environmental tobacco smoke, and mold, can create complex indoor environments with mixed exposures that pose challenges for characterization of the pollutants and identifying the causation of the health outcomes. Therefore, monitoring the indoor air pollutants whether in households or occupational environments via routine and periodical measurements is extremely informative for regulating the indoor air quality. Personal exposure monitors also have been used in several studies to help inform strategies for reducing these exposures. This session will explore the complexity of indoor pollution case studies in both occupational and household environments and will include adverse health conditions related to exposure to indoor air pollutants.
outcomes of some specific pollutants. A discussion will follow by presenting various tools for exposure assessments, including in vivo internal dose assays and a novel aggregate modeling approach. This session aims to address the significance and challenges of assessing indoor air quality due to the diverse nature of indoor pollutants. It will illustrate how the exposure data are incorporated into assessment tools to identify data gaps and inform decisions for regulation, mitigation, and prevention of adverse health outcomes.

1128 Air Pollution Exposure and Health: Filling in the Blanks
J. Thornburg, and R. Chartier. RTI International, Research Triangle Park, NC. Sponsor: E. Mutlu

 Globally, exposure to high levels of air pollution is responsible for an estimated 7 million premature deaths annually with ~ 4 million directly attributable to exposure to household air pollution (HAP). Lack of access to affordable clean fuels makes exposure to HAP a much more pervasive problem in low-and-middle-income countries where some of the most at-risk populations (pregnant women, small children) may be disproportionately exposed to high levels of HAP. In addition to increased mortality, air pollution has been linked to a myriad of adverse health outcomes, both acute (e.g. respiratory infections, cardiac) and chronic (e.g. COPD, cancer). While numerous adverse health outcomes have been associated with air pollution exposure (PM$_{2.5}$ in particular), little is known about the mechanisms by which personal PM exposures can produce systemic effects. The objective of this study was to examine the relationship between personal PM$_{2.5}$ exposures measured by the RTI MicroPEM personal exposure monitor and the relationship with nasopharyngeal carriage of streptococcus pneumoniae, 1) a toxicological study of red oak and cow dung combustion PM2.5 emissions using filter extractions and a cellular model (AS49 and THP-1), and 3) cytotoxicity and gene expression changes of lung epithelial cells exposed to wood smoke at an air-liquid interface (ALI) using a novel ALI exposure chamber. The cumulative findings of these studies contribute to our overall goal of better understanding the impacts of biomass burning-derived emissions, PM toxicity, personal exposure burden, and potential health effects. While these were disparate projects, a combination of these methods, and the measurement tools used therein, in a single study could help bridge the gaps between the fundamental physical and chemical properties of PM that contribute to exposure toxicity (size distribution and composition), the personal exposure measurements used to collect real-world exposure data, and the potential mechanisms that drive the health outcomes of the exposed.

1129 Investigation of Systemic Exposure of Volatile Organic Compounds (VOCs) following Inhalation Exposure: A Case Study with Mixed Xylene Isomers
E. Mutlu. NIEHS/NTP, Research Triangle Park, NC.

The alkylbenzene class of compounds, specifically mixed xylene isomers, were nominated to the National Toxicology Program (NTP) for testing due to its widespread potential for inhalation exposure. Xylene (as a mixture of isomers) is one of the four VOCs in BTEX (benzene, toluene, ethylbenzene (EB), and xylene) and is found in crude oil. Primary route of exposure to xenylens is via inhalation from consumer products (e.g. paint), occupational exposures (solvents), industrial emissions, and automobile exhaust. Benzene and EB which are either carcinogenic and possibly carcinogenic, respectively, in humans are structurally similar to xylene. However, potential adverse effects of xylene are unclear at the present time. Commercially available xylene contain up to ~20% of EB. In order to evaluate the toxicity of xenylens in the absence of EB, a test material was generated by blending individual isomers at a ratio of 2:5:6:24 for ortho meta- para-xylene, which is the ratio found in commercial xylene mixtures. As a part of a larger study investigating the toxicity of the xylene mixture following whole body inhalation exposure in male and female HSD:Sprague Dawley 5D rats and B6C3F1/N mice, blood samples were collected from animals exposed to 0 (control), 150, and 600 ppm (6 h, 5 days/wk, 4 wks at 2 and 2A1 h (rats only) after the last exposure to understand systemic exposure. Each individual isomer increased with the exposure concentration in both rats (~210-4400 ng/mL) and mice (~90-7100 ng/mL) compared to 2 h samples (~1400-4400 ng/mL) for all the isomers. The blood ratios were similar to that in the test material suggesting that the disposition was similar between isomers. In general, assessment of systemic exposure of VOCs is challenging due to analyze volatility and hence stringent measures for sample collection and storage are critical to ensure accurate exposure data are generated. An estimation of systemic exposure is essential to aid interpretation of toxicology studies. This talk is focusing on one example for the feasibility of generating systemic exposure data in a larger program evaluating the toxicity of inhalation chemicals at the NTP.

1130 Exposure, Health Risks, and Control of Volatile Organic Compounds in Nail Salons

Nail salon technicians face chronic exposure to volatile organic compounds (VOCs), which can lead to a range of adverse health outcomes including skin, eye, and respiratory irritation, headaches, lung inflammation, asthmatic exacerbations, neurobehavioral complications, and cancer. Research studies have examined various aspects of nail salon environments including VOC exposure, Occupational Exposure Limits compliance, and ventilation. Our research team measured indoor levels of formaldehyde and the aromatic compounds benzene, toluene, ethylbenzene, and xylene (BTEX) in 6 Colorado nail salons. We also measured personal exposure concentrations for 9 VOCs (BTEX, acetone, ethyl acetate, n-buty acetate, methyl methacrylate and 2-butane). The study determined that the concentrations of some of these compounds were comparable to those measured in studies of oil refinery and auto garage workers. Cancer risk models determined that a 20 yr exposure to formaldehyde and benzene concentrations measured in our study will significantly increase worker’s risk of developing cancer in their lifetime. Our team also characterized VOC emissions from typical nail care products and conducted control chamber studies to investigate the removal of VOCs using low-cost, sorbent sinks (i.e., coco coir, biochar, and activated carbon) and active flows provided by synthetic jets. Additional optimization studies were conducted using a novel, low-cost platter matrix treated with activated carbon (AC) using acetone as the model VOC. These controlled studies determined that VOC removal by sorbent sinks increased with external surface area and with thickness to a lesser extent. Active flow conditions also enhanced VOC removal from the air. Experimental data were then used in nth-order general rate models with VOC-concentration-in-air and VOC-mass-adsorbed as fit parameters. The model results indicated that sorbent sinks provided significant VOC removal in a 1400 m$^3$ nail salon but required approximately 25 m$^2$ of surface area. Estimated removal efficiencies were ~80%, similar to values reported in a previous study. These low-cost sorbent sinks were then adapted into ‘air-cleaning’ art configurations that were informed by nail salon owners and technicians.

1131 Fungal Exposures within the Indoor Environment
T. Croston. NIOSH, Morgantown, WV. Sponsor: E. Mutlu

Fungal contamination found within damp indoor environments negatively affects indoor air quality, and exposure to fungal bioaerosols and secondary metabolites has become an area of great public health concern. No exposure limits exist to protect occupants and workers exposed to these contaminated environments. Assessments of these environments have identified prominent fungal species proposed to contribute to health ailments, such as “Sick Building Syndrome”. Associations between adverse respiratory health effects and exposure to indoor fungal contamination have been identified; however, the physiological and toxicological effects following repeated fungal inhalation exposure have not been fully characterized. An acoustical generator system delivered dry, unmodified aerosolized fungal spores to mice housed in nose-only chambers to mimic the natural route of human inhalation exposure. We used occupationally relevant doses of NTP-nominated fungal species, Aspergillus fumigatus, Stachybotrys chartarum, and Aspergillus versicolor, inhaled by the mice in order to investigate the mechanisms influencing the pulmonary immune responses following exposure. These subchronic exposure studies demonstrated that the pulmonary immune responses are characterized by differing T-cell phenotypes, as well as variable RNA expression and proteomic profiles. While A. fumigatus exposure elicited a mixed T-cell response, S. chartarum and A. versicolor elicited more of a Th2-dominate response following 13 twice-weekly exposures. Histological assessment identified pulmonary inflammation, collagen deposition and tissue remodeling following exposure to all three fungal species independently. Results suggest that the fungal component inducing immune and physiological responses vary between fungal species, such as viability or fragmentation of the fungal test article or the influence of secondary metabolites. Additional studies are warranted, including mixed fungal exposures and utilization of in vitro systems, to advance the understanding of the mechanisms that contribute to the
physiological and toxicological responses that follow fungal exposure. Such studies may lead to improvements in biomarker identification, as well as the contribution to establishment of recommended exposure limits.

1132 Prediction of Population Exposures to Chemicals in the Indoor Residential Environment


The emissions of chemicals from indoor, or “near-field”, sources such as building materials and household articles contribute significantly to human exposure. These chemicals may include plasticizers (e.g., phthalates), flame retardants, synthetic fragrances, environmental phenols, and other volatile or semivolatile compounds. Many of these chemicals have been investigated for association with various health endpoints including endocrine disruption and asthma. Unfortunately, existing sources of hazard and exposure data do not directly address many thousands of chemicals that may be present in the indoor environment or used in commerce. This talk will focus on high-throughput methods being developed by the EPA Office of Research and Development to characterize human near-field exposures to these chemicals. Models and analytical approaches are being developed to address critical gaps in relevant information required to predict chemical occurrence, emission, fate, and ultimately human exposure in indoor environments. Informatic methods are being used to extract reported chemical ingredient information from thousands of public documents (e.g., SDS sheets, ingredient list, manufacturer disclosures) while new non-targeted analytical methods are being used to screen for thousands of unreported chemicals in consumer products. These data are being integrated with product sales information for U.S. households to identify critical co-exposures having common endpoints (e.g., endocrine disruption) that may ultimately increase risk. In addition, new structure-based models are being developed to predict critical parameters for estimating chemical emission from consumer articles used indoors. Finally, the talk will cover the development of population-based screening-level and mid-tier models for predicting indoor fate and transport of chemicals to air and dust that result from chemical release.

The high-throughput exposure estimates generated via these methods can be integrated with in vitro hazard information (assay results or models for bioactivity) to develop screen-level metrics of risk. Overall, these high-throughput methods will allow the identification and further testing of those chemicals which are more likely to pose a risk to humans.

1134 Recent Progress toward Reducing Animal Use and Adopting New Approach Methods

A. Lowit. US EPA, Washington, DC.

In a September, 2019 directive, EPA’s Administrator Andrew Wheeler calls for the agency to pursue a reduction in animal testing. The memo states, EPA will reduce its requests for, and funding of, mammal studies by 30% by 2025 and eliminate all mammal studies and future Office of Chemical Safety and Pollution Prevention recently has made progress towards reducing animal use and adopting new approach methods (NAMs). This presentation will provide examples related to granting waivers for mammalian studies for a variety of study types such as inhalation and genotoxicity studies and for ecotoxicology studies in fish and birds, including a recent waiver guidance published in 2020 for some bird studies. Advances in NAMs and use of pharmacokinetic information in dose setting for use in cancer and chronic toxicity testing will be discussed. EPA has also recently announced implementation of QSARs (e.g., https://ntp.niehs.nih.gov/whatwe-study/niceatm/test-method-evaluations/comptox/ct-opera/opera.html) and computational approaches for endocrine disruption of pesticide active ingredients and inerts. In addition, EPA’s Office of Pesticide Programs released the first ever human health risk assessment using in vitro studies used as points of departure for quantitative risk assessment (https://www.regulations.gov/ document?D=EPA-HQ-OPP-2014-0159-0008). Specifically, in collaboration with the National Toxicology Program, EPA has used skin sensitization in vitro studies coupled with artificial neural network-based defined approach (DA) to determine points of departure used in the isothiazolone risk assessment instead of using laboratory animal data to evaluate risks for dermal sensitization.

1133 Mind the Gap: Finding Practical Ways to Fast-Track the Future of Animal-Free Toxicology Testing

A. Lowit. US EPA, Washington, DC.

In recent years, the march toward an animal-free future for safety assessment has accelerated and now seems within reach. However, in the agricultural, chemical, and pharmaceutical sectors, animal studies are still quite heavily relied upon to characterize the hazard and risk profile of a new chemical or product. Where regulations currently demand the generation of animal data, performing parallel assessments using nonanimal methods will help to bridge the gap and fast-track the animal-free future of toxicology testing. This Symposium aims to be a practical session to provide guidance and shared examples that can bridge the current gap, culminating in a panel session where recommendations can be discussed and explored. For example, good-quality, integrated kinetic data and predictions could provide a wealth of information that could be used for IVIVE, setting up in vitro assays at relevant concentrations, and increase confidence in the safety assessment. Integrated mode-of-action investigations could provide better information on the mechanism of effect seen in animals and their potential human relevance. This will require a concerted effort between different stakeholders and the adoption of modern and common practices to testing and assessment. This Symposium aims to consider what the agricultural, chemical, and pharmaceutical sectors can learn from industries that are already operating in an animal-free environment, and how future technologies can help to usher in a truly animal-free toxicity testing future. In presentation 1, Dr. Lowit will present the perspective of a regulatory agency, the US EPA, who have set an ambitious target for reducing animal use and explain options open for waiving animal studies and using new approach methodologies (NAMs). In presentation 2, Dr. Terry will present an overview of a modern agricultural product’s mammalian toxicology program, where animal studies are still heavily relied upon but where avenues have been taken to fully embrace and implement the 3Rs replacement, reduction, and refinement) and NAM approaches wherever possible. Dr. Dent will then present experience and learnings from the cosmetic industry, which has been “animal-free” for many years and leads the field in application of NAMs. Finally, Dr. Boekelheide will summarize the learnings and present a vision for “bridging the gap” between a reliance on animal data and the animal-free future of toxicity testing. After each of these 30-minute presentations (with five minutes each for questions), there will be a 25-minute panel discussion that will be moderated by Dr. Sewell and will be a practical session to discuss examples of real use presented in the previous talks. Key examples will be identified by speakers ahead of time for the panel discussion and pre-agreed “characteristics” will be shared with the different sectors. For example, perhaps methods in integrated toxicokinetics could be better or differently applied in different sectors. How could this help to speed the adoption of animal-free approaches, and what are the benefits, costs, and challenges associated with the different sectors? An important feature of the discussion also will be to further explore what the different sectors can learn from each other in the application of nonanimal approaches. The output from this panel session will form a publication in the peer-reviewed literature to widen the audience and discussion even further.

1135 Advancing Safety Assessment in the Crop Protection Sector: Building the Bridge to an Animal-Free Future

C. Terry. Corteva Agriscience, Indianapolis, IN.

A paradigm shift is underway in chemical safety assessment with the potential to greatly reduce reliance on animal testing in favor of in vitro methods and other New Approach Methodologies (NAM). While the science and technology supporting use of such methods has advanced tremendously in recent years, animal testing is still considered the ‘gold standard’ for regulatory decision-making, particularly in the crop protection sector. Even so, there are multiple innovative strategies that can be employed to reduce animal use without compromising the integrity or utility of the information needed for safety determinations. First, toxicological endpoints should be integrated to the extent possible to reduce animal testing. For example, neurotoxicity, immunootoxicity and genotoxicity endpoints can be readily assessed in a single 90-day rodent toxicity study, thus eliminating the need for stand-alone studies to address these endpoints. In addition, generation of toxicokinetic data (without use of satellite animals) provides important internal exposure information to contextualize organ toxicity as well as key information for selection of relevant dose-levels. Further, when toxicological findings are observed, mechanistic in vitro assays can be employed to investigate the mode of action and human relevance of the effects, thus reducing the need for follow-up testing and providing key risk management information. Finally, in vitro methods—often in combination (e.g. defined approaches) - are increasingly being implemented as replacements for acute toxicity endpoints, currently conducted in vivo. Further adoption of NAMs for regulatory decision making will require continued cooperation with multiple stakeholders working together to bring forward and implement relevant and health-protective approaches.
Tolerated Dose (MTD). Recently, incorporating kinetic data to support the sed-dose toxicity studies are guided by the consideration of the Maximum treatments and minimize animal distress. Conventionally, top doses in repeat-enhance interpretability of study results used in human health risk assess- appropriate doses in animal toxicity studies, especially top-dose levels, willment in assessing and informing the safety of chemicals or drugs. Selecting how progress in the cosmetics industry might inform the development of non-animal approaches in other sectors.

**1137 The Path Forward to a Near Future That Relies Primarily on Nonanimal Tests for Safety Assessment**

K. Boekelheide. Brown University, Providence, RI.

Remarkable progress has been made since the release of the National Research Council’s report in 2007, “Toxicity Testing in the 21st Century: A Vision and a Strategy.” At the time of release, there was vocal skepticism that safety testing was even possible without animals providing an integrated model of mammalian physiology. While there is still much work needed to assure safety with non-animal models and approaches being developed, there is general agreement that using the best science will get us to this goal. What are the major challenges? What are we missing? How do we bridge the gap between an animal-based testing past and a non-animal-based testing future? This talk will address two major challenges to bridge this gap by discussing the following concepts: (1) Adversity. In silico and in vitro determination of an adverse effect is a major challenge, since traditional tools used in animal-based testing to determine adversity - histopathology/apical endpoints (e.g., body weight) - are not available. The relative strengths and weaknesses of in vitro autonomous determinations of adversity will be com-pared to approaches that reference apical endpoints (e.g., adverse outcome pathways). The concept of “in vitro pathology” will be introduced as a tool for determining adversity through the integration of morphological, molecular, and functional endpoints. This will conclude with a proposal to use different definitions of adversity for safety decisions depending upon regulatory context. (2) Metabolism. While parent chemical metabolism has always been an issue in animal studies because of the required species extrapolation for human relevance, it is particularly obvious as a problem for in vitro models. The path forward to incorporating metabolism into in vitro systems will be discussed, including use of human hepatocyte models coupled with target cell type and organoid exposures. An important question to ask: What reg-ulatory contexts require sophisticated knowledge of metabolism? Finally, a general strategy will be proposed to make the future of non-animal based safety testing a reality by combining case studies of well-understood model compounds and preclinical settings, educational efforts among the regulators/the regulated, and complimentary data packages of animal and non-animal safety assessments for novel compounds.

**1138 Opportunities and Challenges in Utilization of Toxicokinetic Data in Dose-Level Selection for Repeated-Dose Toxicity Studies**

J. Domoradzki. Corteva Agriscience, Indianapolis, IN.

Understanding and quantifying dose-response relationships is a critical ele-ment in assessing and informing the safety of chemicals or drugs. Selecting appropriate doses in animal toxicity studies, especially top-dose levels, will enhance interpretability of study results used in human health risk assess-ments and an awareness of all distal toxicokinetic details. Currently, top-dosedose toxicity studies are guided by the consideration of the Maximum Tolerated Dose (MTD). Recently, incorporating kinetic data to support the se-lection of top dose in repeated-dose toxicity studies for environmental chemi-cals, such as the Kinetically Derived Maximum Dose (KMD), has been a topic of intense international debate. KMD refers to the dose at, or slightly above, the deviation from linear kinetics based on blood levels of the parent compound or significant metabolites. There is little value in setting a top dose higher than the dose at which absorption is saturated, since systemic dose does not increase with increasing exposure. On the other hand, when a detoxification process, such as biotransformation or excretion, is satu-rated, toxicity data generated by doses much higher than saturation may not be predictive of health risks resulting from much lower environmental exposures. In the pharmaceutical field, kinetic information is routinely consid-ered in preclinical testing, and for chemicals, a limiting dose is a common criterion for dose selection. In the second talk, the “how” and “when” to use kinetics in dose selection will be described throughout mammalian toxicology studies submitted for registration of ag-rochemicals. The second presentation offers the regulatory perspectives on the role nonlinear kinetics play in reviewing preclinical studies in drug applica-tion packages. The third presentation will highlight commonly raised challenges related to the KMD concept, such as determining an inflection point from sparse kinetic data or lacking exposure data to compare with doses used in repeated-dose toxicity studies. The first presentation will describe the significance of both toxicokinetic and toxico-dynamic processes for observed dose-response relationships. In the second talk, the "how" and "when" to use kinetics in dose selection will be described for environmental chemicals. The third presentation offers the regulatory perspectives on the role nonlinear kinetics play in reviewing preclinical studies in drug applica-tion packages. The fourth presentation will describe the significance of both toxicokinetic and toxico-dynamic processes for observed dose-response relationships. In the second talk, the "how" and "when" to use kinetics in dose selection will be described for environmental chemicals. The third presentation offers the regulatory perspectives on the role nonlinear kinetics play in reviewing preclinical studies in drug applica-tion packages. The fourth presentation will describe the significance of both toxicokinetic and toxico-dynamic processes for observed dose-response relationships. In the second talk, the "how" and "when" to use kinetics in dose selection will be described for environmental chemicals.

**1139 The Role of Toxicokinetics and Toxicodynamics in Dose-Response Studies**

H. Barton. Independent Consultant, Mystic, CT.

The observed dose-response in toxicity studies results from the combined effects of toxicokinetic and toxicodynamic processes in the selected animal species, strain, sex and life stage over the duration of the study. The number of animals per dose group and the number of dose groups can impact the de-tection limit for effects and the evaluation of the mathematical dose-response relationship. Toxicokinetic processes include absorption (influenced by the chosen matrix and vehicle), distribution, metabolism, and excretion; toxicody-namic processes impact biological functions starting from target molecules moving on to cells to organs to whole systems, determining whether a parent compound and/or a metabolite can cause toxicity. In some cases, the dynamic processes alter the toxicokinetics, for example, by inducing metabolizing en-zymes or depleting key protective molecules in cells. Safety assessment for pharmaceuticals typically rely on measurements of internal concentrations, usually plasma concentrations, but this has less consistently been applied for environmental chemicals. Dose-dependent transitions in toxicodynamics (mode of action) often appear key to determining toxicity study outcomes that are not human relevant or poorly extrapolated to humans. In these cases, saturation of a toxicokinetic process alone cannot be used to select high dose in repeated-dose toxicity studies. Rather, selection of the high dose should consider multiple factors addressing exposure, toxicokinetics, and toxicody-namics. And, extrapolation to humans of observations in experimental an-imals can be improved by characterizing the similarities and differences in kinetics and dynamics (also referred to as mode of action for environmental chemicals), such as by applying frameworks for assessing human relevance and mathematical models.
1140 Informing Toxicity Study Design Using Kinetic Data: From Beginning to End

J. Domoradzki, Corteva Agriscience, Indianapolis, IN.

Toxicokinetic (TK) integration into toxicity studies starts with determining kinetic parameters early in the safety testing program. Probe ADME (absorption, distribution, metabolism, and excretion) data are collected over time to assess the relationship of blood, plasma and urine samples to determine appropriate biomarkers (parent and major metabolites) to be monitored in future repeated-dose toxicity studies. In repeated-dose studies, TK data are generated at steady state to understand the systemic exposure to the test material. The integrated TK data obtained across toxicity studies follows 3Rs principles (without the use of additional/satellite animals) and provides critical information to understanding differences in response across doses, species, strains, sexes, and life stages. In cases where nonlinearity of the dose–response curve can be identified, the high doses for the subsequent longer-term studies can be selected based on the nonlinearly derived maximum dose (KMD) and apical endpoints. Determining the KMD has been the subject of international debate and examples of determining dose proportionality (AUC vs. dose) will be presented. 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 (sulfafloxacin), parent and 1 metabolite (halaluxifen-methyl and –acid, florpypyrauxifen-benzyl), parent and 1-2 metabolites (fenpropicamid). Knowledge of 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. 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.

1141 Perspectives of Kinetic Considerations in Drug Applications

J. Hawes, US FDA, Silver Spring, MD.

Regulatory agencies apply considerable scrutiny to methods and supporting data for proposed clinical doses. In this presentation, regulatory perspectives on the roles that non-linear kinetics play in regulatory review of drug application packages will be discussed, including the relationship with toxicological findings in the review of preclinical studies that are conducted with dose selection criteria outlined in the OECD Test Guidelines (TG) or ICH Guidelines. For example, OECD TG116 suggested that top dose selection based on infection points in nonlinear kinetics indicates that metabolic saturation represents an equivalent indicator of biological stress, and, ICH S3A recommends that when kinetic data indicate saturation of absorption, the lowest dose of the substance producing the maximum exposure would be accepted as the top dose. Preclinical studies are used to determine the human equivalent dose (HED), determine the top dose, and determine toxicities that need to be monitored for in clinical trials. The influence of non-linear kinetics on strategies for determining the starting dose, dose escalation, and maximum recommended human dose (MRHD) in clinical trials based on preclinical data will be discussed to provide insights on ancillary data required to support the TK data. Limitations that have been noted in the KMD approaches submitted so far for regulatory purposes will also be presented, for example, lack of information on metabolites, insufficient number of doses in TK studies, along with the steps that the Agency is taking to address these limitations. The latter includes actively participating in an international symposium designed to provide additional guidance to interested stakeholders, including Industry, on the use of KMD for regulatory purposes.

1142 Case Studies: Considering Nonlinear Kinetics in Dose Selection and Interpretation of Animal Toxicity Studies

J. Bus, Exponent, Midland, MI.

The widely used non-carcinogenic herbicide 2,4-dichlorophenoxyacetic acid (2,4-D), an example of a peripheral organ, organ transplanted (OPT) in rats, will be used to illustrate how consideration of onset of nonlinear toxicokinetics provides valuable a priori insights into selection of top doses in in vivo animal toxicity studies that are relevant to real world human risks, and also inform post hoc interpretation of the human relevance of high dose specific non-cancer findings of animal toxicity studies. For 2,4-D, saturation of renal clearance results in onset of target organ models of action that are not quantitatively relevant to human due to high-dose specific altered distribution associated with decreased plasma protein binding and resulting altered dosimetry to and within target organs such as brain. Importantly, consideration of the KMD is critical in that use of conventional MDI-based top dose selection identifies hazard responses that are not quantitatively appropriate for risk assessment. This case will also demonstrate how in vivo toxicokinetic nonlinearity informs a prior design and/or post hoc risk relevance evaluations of in vitro hazard and/or mode of action investigations, i.e., in vitro concentrations above a KMD do not inform human hazard or risk since resulting systemic plasma/tissue concentrations are not encountered following realistic human exposures. The case of the chemical intermediate and fuel constituent ethylbenzene will also be presented as an example of how post hoc consideration of nonlinear toxicokinetics driven by saturated liver P450 metabolism informs the human risk relevance of rodent tumors observed only at the high saturating dose. Such metabolic saturation is readily signaled by dose-disproportionate increases in the fraction of parent ethylbenzene detected in exhaled air. In conclusion, these cases offer examples of experimental approaches facilitating selection of test doses (concentrations) that ultimately improve the human risk relevance of in vivo and in vitro hazard identification studies and mode of action evaluations.

1143 Canadian Regulatory Perspective: Consideration of Toxicokinetic Data in Dose Selection for Pesticide Toxicity Studies

C. Adcock, Health Canada, Ottawa, ON, Canada. Sponsor: J. Domoradzki

Pharmacokinetic (PK) and toxicokinetic (TK) data are routinely part of pest control product applications; however, in recent years, Health Canada’s Pest Management Regulatory Agency (PMRA) has started to receive pest control product applications that propose the selection of the top dose in a toxicity study based on pharmacologically derived maximum dose (KMD). An underlying intent of using the KMD is to provide more precision for the toxicity data used for risk assessment purposes, which includes dose selection. Using afidopyropen and broflanilide, two recently published health risk assessments that proposed the use of KMD, PMRA will provide a general overview of the Agency, followed by the types of data required for the registration of a pest control product in Canada. A specific focus will be on the toxicity studies that are required to establish the dose for risk assessment purposes and the role for TK data. Limitations that have been noted in the KMD approaches submitted so far for regulatory purposes will also be presented, for example, lack of information on metabolites, insufficient number of doses in TK studies, along with the steps that the Agency is taking to address these limitations. The latter includes actively participating in an international symposium designed to provide additional guidance to interested stakeholders, including Industry, on the use of KMD for regulatory purposes.

1144 Testing the Waters: How the Zebrasfish, Xenopus, and Medaka Models Are Advancing Our Understanding of Reproductive and Developmental Toxicity

J. Plavicki, Brown University, Providence, RI.

The power of toxicological research relies on our ability to accurately assess the impact of toxicant and pharmacological exposures on an organism’s development and function. While in vitro assays for detecting chemically induced changes in behavior, disease or dysregulation of critical pharmacological agents during sensitive windows of development. The first speaker will describe how transgenic zebrafish lines have been leveraged to screen 300 Phase I ToxCast chemicals and, subsequently, identify chemical disruptors of skeletal, vascular, or neurastomorphogenesis. The second speaker will focus on the use of Xenopus and medaka models for conducting predictive and mechanistic toxicology studies to prioritize the testing of existing compounds and aid in the development of safer, novel commercial products, as well as determine the appropriate usage of critical pharmacological agents during sensitive windows of development. The second speaker will describe how transgenic zebrafish lines have been leveraged to screen 300 Phase I ToxCast chemicals and, subsequently, identify chemical disruptors of skeletal, vascular, or neurastomorphogenesis. The third speaker will discuss how multiple aquatic models can be used in conjunction with in vitro assays to predict the endocrine-disrupting potential of novel compounds and, thus, establish critical developmental windows. The fourth speaker will describe how different genetically encoded calcium indicators (GECIs) can be used to perform functional neuroimaging in zebrafish and how GECIs can be used to determine which brain regions and neuronal subtypes are impacted by toxicant exposures. Finally, the Symposium will end with an overview of how zebrafish can be used to determine the developmental window in which antiviral drugs can be safely be used during pregnancy to prevent HIV transmission. Together, the talks in this Symposium demonstrate the diverse ways in which aquatic models are being used to further our understand-
ing of chemical toxicity, predict adverse outcome prior to chemical exposure in humans, understand the impact of neurotoxicants on brain function, and optimize the safe use of pharmacological agents to treat devastating diseases.

1145 A Pipeline for Prediction of Developmental Toxicants; Screening in Zebrafish and Correlation to ToxCast Data

M. Bondesson, Indiana University, Bloomington, IN.

Transgenic zebrafish embryos with tissue- or organ-specific fluorescent reporters can be used to visualize normal and perturbed development of those tissues in ways not possible with larger vertebrates. We exposed transgenic zebrafish embryos to the Phase I chemicals from EPA’s Toxicity Forecaster (ToxCast) program to visualize malformations in the skeleton, vasculature, and in neurornast (mechanosensory cells). Around 300 chemicals were screened and 38 skeletal, 10 vascular and 22 neurornast disruptors were identified. We used these distinct sets of developmental disruptors and univariate correlation analysis to identify ToxCast assays that were affected by the same chemicals. Identified assays included those that measure vitamin D3 metabolism and dopamine transporter activity, oxidative stress and nuclear receptor signaling, and serotonin signaling, for skeletal, vascular and neurornast disruptors, respectively. We further used the identified assays to make predictions about other chemicals, tested in the Tox21 screening project, but not tested in zebrafish embryos, using the Toxicological Priority Index (ToxPi) program. This program ranks compounds based on their AC50 value for the selected assays. Predicted disruptors were tested in the laboratory, and several new developmental toxicants were identified. We conclude that medium throughput screening data from an in vivo aquatic model in combination with modeling of ToxCast high throughput in vitro data can be used to produce testable hypotheses on modes of action of chemical exposures, and to predict tissue specific malformation of chemical exposures. Disclaimer: This abstract does not necessarily represent US EPA policy.

1146 Combined Approach of In Vitro and Aquatic Models to Predict Developmental Toxicity and Endocrine Disruptors

E. Bianchi, Corteva Agriscience, Indianapolis, IN.

The discovery and development of novel molecules and products is a complex process that traditionally relies on intensive animal use for hazard identification. There is growing interest in the possible health threat posed by endocrine-disrupting chemicals (EDCs), and the failure to predict hazardous endpoints such as endocrine disruption remains a critical challenge for discovery programs. Embracing high-throughput predictive models for assessment of off-target hazardous properties of molecules early in discovery can aid in the development of human health and environmentally friendly products. In this presentation, we will discuss the predictive utility of a combined approach of aquatic models, including medaka and transgenic Xenopus and zebrafish models, together with in vitro approaches focused mainly on the estrogen, androgen, thyroid, steroidogenesis, and retinoic acid pathways to predict developmental toxicity and endocrine disruption. Case studies will be used to illustrate how high-throughput in vitro screening combined with reduced animal testing in aquatic model systems can be leveraged for molecule design. For example, high-throughput aromatase inhibition screening combined with wild-type and transgenic medaka studies were successfully employed to eradicate off-target toxicity of aromatase inhibition and endocrine disruption properties for a class of novel compounds. Additionally, we will discuss the utility of harnessing transgenic zebrafish and Xenopus models for predictive thyroid disruption for direct-acting modes of action (i.e. thyroid peroxidase inhibition) for both ecotoxicology and mammalian toxicity applications. We conclude that the combination of in vitro and aquatic models provides a valuable platform for the prioritization and design for novel compound development.

1147 Larval Zebrafish Neurodevelopmental Toxicity Testing and Variables That May Affect the Outcome


The US EPA is evaluating alternative methods to screen and prioritize chemicals for developmental neurotoxicity. Specifically, we are using the zebrafish (Danio rerio) light/dark locomotor activity test, a behavioral paradigm which simultaneously tests individual 5-6-day-old zebrafish larvae under both light and dark conditions in a 96-well plate using a video tracking system. This for-

1148 Using Genetically Encoded Calcium Indicators to Understand How Developmental Neurotoxicants Affect Neuronal Function

N. Martin, Brown University, Providence, RI.

Researchers have utilized the throughput capabilities of the zebrafish model to detect the neurotoxic potential of a large number of compounds. These powerful screens can provide a behavioral readout of dysfunction and identify gross morphological changes, but do not identify the brain areas or neuronal sub-types that mediate the observed behavioral changes. Here, we describe how genetically encoded calcium indicators (GECIs) can be used to perform functional neuroimaging in vivo in zebrafish to identify how specific neurotoxicants can affect brain health. To visualize changes in brain activity following toxicant exposure, we are using the CaMPARI transgenic line. CaMPARI, which stands for Calcium Modulated Photoactivatable Ratiometric Integrator, is a photo-convertible protein that permanently converts cells with high calcium from green to red in the presence of 405 nm light. Since neuronal firing is accompanied by an influx of calcium more active cells appear red following photoconversion, while less active cells remain green. To photoconvert CaMPARI in freely behaving fish, we modified our Noldus DanioVision behavioral unit to include a 405 LED. As an initial proof-of-concept experiment, we acutely exposed zebrafish larvae to either (1) the GABA-inhibitor pentylenetetrazol (PTZ), (2) the anesthetizing agent MS-222, or (3) untreated embryo rearing medium. As expected, PTZ exposure increased motor activity and calcium signaling, whereas the acute exposure to MS-222 decreased motor behavior and neuronal activity. After generating an experimental pipeline for photoconversion and analysis, we exposed larvae to varying concentrations of perfluorooctanesulfonic acid (PFOS), a pervasive toxin that has been shown to produce hyperactivity in larval zebrafish. Chronic exposure to PFOS resulted in a significant global increase in neural activity and either hyper- or hypopac-tivity depending on the dose. We are currently examining the effects of other known neurotoxicants such as dioxins and non-dioxin-like polychlorinated biphenyls on neuronal activity. We are complementing our whole brain and regional CaMPARI studies with studies using GccMP, a GECl that allows for the visualization of single-cell calcium dynamics. Together, our functional neuroimaging studies paired with our behavioral analyses will provide insight into how neurotoxicants affect brain function and health.

1149 Using Zebrafish to Identify Mechanisms of Pharmaceutical Toxicity

D. Gorelick, Baylor College of Medicine, Houston, TX.

Dolutegravir (brand name Tivicay) is a widely prescribed antiretroviral medication used to treat HIV infection. Epidemiologic studies in Botswana found an association between pregnant women taking dolutegravir and increased incidence of neural tube defects in their offspring. Folate deficiency increases the risk of neural tube defects. We tested whether dolutegravir exposure caused toxicity in zebrafish embryos, and whether toxicity could be rescued by folate. We found that zebrafish embryos exposed to dolutegravir prior to gastrulation exhibited malformations or death by 24 hours post fertilization, a developmental stage when many organs have differentiated. We found that dolutegravir exposure was toxic during a critical window of development prior to gastrulation. This is similar to what was observed in humans, where there was no increase in neural tube defects in the offspring of preg-nant women that began taking dolutegravir in the months after conception. Co-administering folate together with dolutegravir rescued toxicity in zebrafish embryos. Dolutegravir blocked folate binding to folate receptor (FOLR1) in vitro. Our results suggest that dolutegravir causes neural tube defects by...
Can We Improve Clinical DILI Risk Prediction Using In Vitro Toxicokinetic Assays in Combination with Available Physical Chemical Properties and In Vitro Safety Assays?

J. Jackson. Pfizer Inc., Groton, CT.

Epidemiological drug-induced liver injury (DILI) studies and predictive models have shown dose as an important factor in determining the risk of adverse drug reactions in the human population. Drugs with daily dose ≥ 50 mg have a higher frequency in DILI severity. Although there is evidence daily dose may be an adequate discriminator between high and low DILI risk compounds, other evidence suggests it does not adequately reflect hepatic exposure making incorporation of this parameter into an early predictive DILI screening strategy problematic. For instance, 43.8% of “No DILI” drugs would be predicted as “Not Safe” regarding hepatotoxicity potential at a 50 mg/dy dose threshold resulting in the loss of many good drug candidates. Therefore, we hypothesize adding toxicokinetic assays including in vitro measures of plasma protein binding, blood plasma ratio, and hepatic partitioning (Kpu) coefficient to refine daily dosage into hepatic exposure would improve DILI prediction accuracy when combined with physical chemical properties and safety assays. Our data will demonstrate our proposed strategy’s capacity to discriminate high from low DILI risk compounds.

Application of Machine Learning to Determine Genotoxicity Mode-of-Action Classification Based on a Multiparametric Flow Cytometry Assay

J. Remis. Litron Laboratories, Rochester, NY.

The advent of flow cytometric instrumentation that is compatible with high density microtiter plates has led to the ability to develop high content assays that can deliver thousands of datapoints in a single experiment. This often results in “a data deluge” that can readily overwhelm standard the data analysis approaches that are typically employed in flow cytometric analytics. This was the case during the development of the MultiFlow DNA Damage assay which combines multiple biomarker response measures taken at two timepoints in cultured cells grown, exposed, processed and analyzed in 96 well plates. As a means for dealing with the large amount of data produced and the desire to extract more than just a yes/no call for genotoxicity, we created a supervised machine learning approach that categorizes test articles into the following modes of genotoxicity: clastogenicity, aneugenicity and non-genotoxicity. To do this we utilized a training set of compounds with known mode of action (MoA) processed through the MultiFlow method, then analyze using three machine learning models: logistic regression, artificial neural networks, and Random Forest. The outputs from these models were interpreted via a majority-vote ensemble approach and specific probability criteria were established to achieve 94% concordance with a priori MoA classification. Additional testing of the system included leave-one-out cross-validation along with examination of a test set of compounds unknown to the model, further confirming excellent performance. Based on this initial work, the MultiFlow assay has been reduced to practice in both kit and fee-for-service formats. We continue to iterate upon the existing methodology and are now exploring use of area under the curve for data reduction, unsupervised clustering for evaluation of new biomarkers and analytical strategies, as well as high throughput/high content approaches that go beyond MoA to the actual molecular targets of genotoxic agents. Such method development work would be impossible without the utilization of the multitude of machine learning and computational approaches that are available today.
tion-response data for up- and down-regulation across all hormones was used to profile chemical-mediated effects into putative modes of action for chemical-elicited steroidogenesis disruption. Furthermore, Bayesian modeling was used to predict the probability of enzyme inhibition at specific steps in the steroidogenesis pathway leveraging single concentration screening data for use in mechanistic interpretation. Finally, we will also summarize a novel prioritization method based on the Mahalanobis distance reduced the 11-dimensional data to 1-dimensional via calculation of a mean Mahalanobis distance (mMD) at each chemical concentration screened for all hormone measures available. All of these approaches not only integrate complex pathway-level data from this multiplexed assay, but also inform on putative modes of action and provide an uncertainty measure associated with the predicted outcomes.

1156 Across the Life Span: Emerging Mechanisms of Prenatal and Transgenerational Toxicity

J. L. Iulce, University of Arizona, Tucson, AZ.

Toxic perturbations during early windows of susceptibility and the subsequent ontological impacts define broad theories of toxicity and are generally supported by epidemiological studies. However, mechanistic differences often fail to consider variability within affected populations, including sex, genetics, and epigenetics. Specifically, in recent years, the National Institutes of Health have escalated guidelines pertaining to the use of specific sexes in biomedical research involving animal models. As such, the goal of this Symposium Session is to expand upon existing understanding of development and transgenerational origins of disease, introducing additional variables that influence the trajectory of toxic effects. Every year, the Postdoctoral Assembly (PDA) and Graduate Student Leadership Committee (GSLC) propose a Scientific Session to highlight significant contributions of trainees, allowing the opportunity to share cutting-edge research via an oral presentation to internationally recognized experts in toxicology. Three trainees will tackle this topic by presenting their research that centers around prenatal and early-life exposures to environmental contaminants—metals and phthalates—each evaluating a different factor that alters canonical ontological toxic effects. First, combined effects of pre- and postnatal cadmium exposure and postnatal high-fat diet will be explored. This work will introduce the audience to both adverse effects of prenatal metal exposure, as well as sexually dimorphic effects due to poor diet. Next, the effects of phthalates on female reproductive aging will be presented. This work demonstrates not only that environmental phthalate exposure elicits sex-specific effects, but also that these prenatal exposures elicit transgenerational effects. Finally, the influence of specific non-coding RNAs—an emerging paradigm in toxicopigenetics—on neurodevelopment will be introduced, as well as how disruption of this system by lead may selectively interfere with neurodevelopment. Overall, in addition to providing a valuable platform for trainee oral presentations, this session will stimulate conversation around new variables that determine toxicological effects across the life span.

1157 Sexual Dimorphism of Cadmium-Exacerbated Diet-Induced Liver Disease

J. Young, University of Louisville, Louisville, KY.

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver diseases affecting more than 25% of the world’s population. Although obesity is a major risk factor for NAFLD, it does not account for all cases, suggesting the contribution of other factors such as sex and environment exposures. Exposure to the non-essential metal cadmium is implicated in the development of several metabolic diseases, including NAFLD; however, the ability of early-life, in utero Cd exposure to influence the developmental course of obesity-induced NAFLD is poorly understood. Additionally, studies do not consider that such environmental exposures may be life-long and multigenerational. Therefore, we developed an in vivo model of perinatal Cd exposure utilizing CD1 mice, which allowed for postnatal high-fat diet (HFD) feeding. Adult male and female C57BL/6J mice fed normal diets (ND) were exposed to 0 or 5 ppm Cd-containing drinking water for 14 weeks before breeding. At weaning, offspring (F1) were fed ND or HFD and continuously exposed to the same drinking water regimen as their parents. Water consumption and body weights were recorded weekly. DEXA scan technology was used to assess changes in body fat composition and intrahepatic glucose tolerance tests were performed to aid in characterization of insulin resistance. Both male and female mice were sacrificed 24 weeks post-weaning. In both sexes, HFD increased body weight and body fat at a greater rate in males. Exposure to Cd increased HFD-associated weight gain in males, but not females. In males, exposure to Cd exacerbated HFD-induced liver disease as indicated by plasma transaminases, liver to tibia ratios, histology, and hepatic triglycerides. In contrast, Cd exposure did not alter HFD-induced hepatic outcomes in females. The sexual-dimorphic outcome of these results is supported by epide-
of Pb-associated disruption of transposon DNA methylation in mice and humans, including murine brain, we are examining whether interference of the piRNA system by environmental Pb affects neurodevelopment in multiple in vitro models. This work will assess whether disruption of the piRNA system leads to altered DNA methylation at transposable elements and elsewhere, and if this disruption impacts normal neural differentiation and long-term function.

1160 Nonclinical Safety Toxicology Strategies for the Development of Novel Ocular Biotherapeutics
Q. Huang, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT.

The advent of novel ocular biotherapeutics has revolutionized the treatment of ocular diseases and brought immense medical benefits to patients. While substantial experience was gained on the nonclinical safety toxicology study design, dose selection, endpoint evaluation, and data interpretation to enable clinical development, the nonclinical safety strategies for the development of novel ocular biotherapeutics are still evolving. The goal of this Symposium is to share the advanced sciences and new data involving the key nonclinical toxicology topics regarding the development of antibody and gene/cell therapies for ocular diseases. The industry and US FDA experts will share experience, case examples, and data from the recent years as well as provide thought-provoking strategies. The first speaker will discuss modeling and simulation-based PK-TK prediction and translation, and points to consider for dose selection for drugs administered via intravitreal injection. Ocular inflammation is a common finding in nonclinical intravitreal toxicity studies with biologics. The second speaker will focus on examining types, timing, and translatable aspects of ocular inflammation in nonclinical intravitreal toxicity studies with biologics. Biodegradable polymer-based ocular biopharmaceuticals, a novel way to deliver drugs in the eye, has the potential to maintain effective drug concentrations in the eye for an extended period of time, reducing the need for frequent ocular injections. The third speaker will discuss the challenges and opportunities in the development of biodegradable polymer-based ocular biopharmaceuticals. Gene therapy has proven successful in restoring vision due to congenital mutations and holds great promise for treating a broad range of ocular diseases. The fourth speaker will share firsthand experience in the nonclinical and clinical development of the ocular gene therapy. The final speaker will provide US regulatory perspectives on the nonclinical consideration for cell and gene therapies for ocular products. In the end, a Q&A session will engage the audience on the presented topics for further discussion. Attendees of this session will have a better understanding of the current approaches and strategies in the nonclinical toxicological development of novel ocular therapies.

1161 Toxicokinetic Modeling Approaches to Informing Dose Selection for Intravitreal Biotherapeutics
S. Chung, Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, CT. Sponsor: Q. Huang

A growing number of therapeutic biologics have demonstrated their clinical efficacy and safety in the treatment of ocular diseases. Intravitreal administration of such biotherapeutics is one of the most effective drug delivery options for therapeutic targets in the posterior segment of the eye. However, the knowledge of their toxicokinetics (or pharmacokinetics) is still lacking due to some sample collection challenges associated with the highly invasive injection procedure, which often results in post-injection complications including endophthalmitis. Toxicokinetic modeling and simulation (M&S) is a quantitative framework to enable the characterization of ocular drug disposition and allow the prediction and translation of dosage during toxicity studies. In this talk, we will review several preclinical and clinical modeling approaches to characterizing pharmacokinetics of several marketed intravitreal biotherapeutics. In addition, we will discuss aspects to consider for model-informed dose selection for biologics administered via intravitreal injections.

1162 Ocular Inflammation in Nonclinical Intravitreal Toxicity Studies with Biologics: Types, Timing, and Translatability
H. Booler, F. Hoffmann-La Roche Ltd, Basel, Switzerland. Sponsor: Q. Huang

Ocular inflammation is a common finding in nonclinical intravitreal (IVT) toxicity studies with biologics, where it can significantly complicate the nonclinical development and safety assessment of IVT molecules. Ocular inflammation can negatively affect animal welfare, compromise the ability to repeat dose, hamper visualization of the back of the eye impacting in-life endpoints such as ophthalmic examination, optical coherent tomography (OCT) and electroretinogram (ERG), and produce confounding histopathologic lesions which may be difficult to differentiate from true treatment-related effects. Broadly speaking, nonclinical ocular inflammation can be divided into two types, based on the time of onset – acute inflammation, which occurs within a few hours or the first few days of IVT administration; and late-onset inflammation, generally arising around 2-3 weeks after the commencement of dosing. Here we look into the different types of ocular inflammation observed in IVT toxicity studies with biologics; we discuss the patterns of inflammation, their underlying causes, and steps that can be taken to minimize their occurrence. We evaluate factors influencing the potential translatability of nonclinical ocular inflammation, examining a number of case studies which summarize and compare available nonclinical and clinical tolerability profiles for intravitreal therapeutics.

1163 Development of KSI-301, An Ultra High Molecular Weight Phosphorylcholine Biopolymer Antibody Conjugate, for Treatment of Retinal Diseases by Intravitreal Administration

Intravitreal anti-angiogenic therapy has become the standard of care for retinal neovascular diseases. Therapeutic VEGF-A inhibitors administered by the intravitreal (IVT) route have been approved and demonstrated effectiveness in slowing the progression of these diseases with resulting gains in visual acuity. However, the requirement for monthly or bimonthly administration has imposed heavy treatment burdens on patients and providers. Here we describe the design and evaluation of KSI-301, a high molecular weight Antibody Biopolymer Conjugate (ABC) with extended intracocular anti-VEGF antibody durability. The design of KSI-301 leverages the high biocompatibility of a phosphorylcholine-based biopolymer with a stable covalent conjugation to a full-length high-affinity monoclonal antibody, which targets VEGF-A. This high molecular weight (950 kDa) anti-VEGF ABC therapeutic enables IVT injection with prolonged intraocular residence time to achieve effective dosing intervals beyond three months. In preclinical studies, KSI-301 demonstrated improved inhibition of VEGF signaling, improved stability, and therapeutically advantageous pharmacokinetic properties (e.g., extended half-life in ocular tissues and rapid systemic clearance). In repeated dose toxicity studies in non-human primates, KSI-301 was well tolerated after monthly injections in ocular and systemic studies after IVT injections over 26-weeks and intravenous injections over 10-weeks, respectively. These preclinical studies enabled the initiation of dosing to patients in a Phase 1 clinical study. In the ongoing Phase 1 clinical trial, KSI-301 has demonstrated safety, bioactivity, and extended half-life of intravitreal KSI-301 in eyes with wet age-related macular degeneration, diabetic macular edema, and macular edema due to retinal vein occlusion, providing first-in-human proof that this new ABC medicine can effectively block VEGF for up to 5 months.
dose, and looking at appropriate endpoints that could translate into clinical development. We will demonstrate the appropriate selection of animal models, clinically meaningful endpoints, are based on visual function and functional vision, and the need for combined pharmacology and toxicology studies. Ultimately, sound pre-clinical development can lead to robust data to support the design and execution of a clinical development program for gene therapy that can ultimately lead to therapeutic development for indications with high unmet need.

1165 Nonclinical Toxicology Strategies for Development of Novel Ocular Biotherapeutics

T. Chen. US FDA/CDER, Silver Spring, MD. Sponsor: Q. Huang

The conduct of a clinical trial for an investigational cellular and gene therapy (CGT) product is guided by the Code of Federal Regulations (CFR) Title 21, Part 312 to ensure the safety and rights of subjects in all phases of a clinical investigation. According to 21 CFR 312.33(a)(8), the sponsor is responsible for providing adequate pharmacology and toxicology data to support a conclusion that the proposed clinical trial is reasonably safe to conduct. The basic goals of preclinical studies are to establish the scientific rationale of the planned clinical investigation and to assess the potential toxicities associated with administration of the CGT product. Considerations for preclinical assessments of CGT products are discussed in detail in the ‘Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products’ (released in November 2013) and aspects specific to ocular indications are discussed in the ‘Guidance for Industry: Human Gene Therapy for Retinal Disorders’ (released in January 2020). This presentation will provide an overview of the current FDA/CDER considerations for a preclinical development program designed to assess the safety and activity profile of CGT products intended to treat ocular conditions. In addition, this presentation will also provide a snapshot of the modalities for early communication interactions with FDA/CDER/OTAT.

1166 Innovation in Toxicology Training during Summer Undergraduate Internships

L. Aleksunes. Rutgers, The State University of New Jersey, Piscataway, NJ.

Establishing a pipeline for the next generation of scientists is critical for the advancement and expansion of toxicology. Only a limited number of students are exposed to toxicology through curricula in typical undergraduate science majors. As a result, experiential learning opportunities such as summer internships provide intensive training of undergraduates in toxicology. Typically, summer programs include full-time mentored research in toxicology for up to three months. These experiences cover responsible conduct of research, experimental design, literature evaluation, data interpretation, and scientific communication. Cohort experiences that engage multiple undergraduate students provide the opportunity for networking and other career-directed activities. This session aims to provide five-minute talks by successful summer program directors and principal investigators from academia, government, and industry that highlight innovations in undergraduate student engagement in toxicology research. These programs range from small (two to five) to large (10-25) numbers of students per summer. A “blitz” of short talks will provide tangible examples that individual scientists and programs can apply to designing and developing their own summer research experiences. This interactive session will be particularly valuable to research advisors, program directors, and near-peer mentors (including graduate students and postdocs). Undergraduates who attend this session will gain insight into what features and initiatives they should be looking for in a robust summer training experience. Topics that will be highlighted include innovation in recruitment, resources, weekly programming, peer-peer networking, faculty training, diversity and inclusion, field trips, internships in pharmaceutical companies, and social enrichment. As programs have had to adapt to virtual training as a result of the COVID-19 pandemic, speakers will describe innovative approaches to deliver engaging and meaningful experiences online and how these initiatives may have long-term value in reaching students in remote locations across the globe. Additional attention will be placed on pipelines and partnerships between liberal arts colleges and research universities, as well as long-term sustainability of programs using sound financial models. Opportunities for funding summer internships from federal sources such as NIH and organizations including the SOT Intern Program will be reviewed.

The second half of the session will include a moderated panel discussion of various aspects including recruitment, mentorship, networking, presentation, assessment, matriculation into PhD programs, and long-term tracking. During the panel discussion, attendees are encouraged to ask questions of successful program directors with deep experience in developing and maintaining successful summer internships. Using interactive polling, the Chairs will survey the attendees for topics in which they are most interested and for future sessions on undergraduate toxicology research.

1167 Challenges and Opportunities in Applying Quantitative and Translational Systems Toxicology Models to Drug Safety Testing


Despite extensive preclinical testing, new drug candidates still fail during clinical trials when safety adverse events not detected in preclinical studies occur in human subjects or when humans prove to be more susceptible to effects identified in preclinical studies. Although catastrophic outcomes are rare during clinical testing, high-profile clinical safety failures do occur, illustrating the need to understand translational safety to improve clinical safety. This Symposium highlights the use of quantitative systems toxicology (QST) modeling approaches to improve translational safety assessment. Translational safety depends on predicting an outcome in one species based on testing in another and thus represents a fundamental problem in evolutionary biology. Systems biologists have developed models, including modular and network models, to assess the preservation of biology across scales of evolution. These studies create an opportunity to apply similar methods to modeling complex biological systems in a quantitative framework for safety predictions based on convergence (i.e., preservation) or divergence of biochemical and biological pathways relevant to drug action. To advance the application of QST modeling, these novel modeling approaches need to be merged with existing PBPK modeling methods to link drug exposure to pathogenesis in a multi-scale QST framework. An ideal QST framework will incorporate the exposure prediction and the concept of preservation across species, preclinical to human, for the underlying metabolic and stress response pathways that underpin a mechanism of action. Constructing multi-scale QST models across scales of complexity spanning cells to intact organisms represents both a significant opportunity and a significant challenge. For example, testing molecules in advanced human microphysiological systems provides data that can support models of compound exposure and cellular stress response. Transcriptomic and metabolomic data can inform activation of stress response networks and global metabolic models, respectively, linked to metabolism and disposition of a novel drug candidate within an adverse outcome pathway that spans molecular responses to pathogenesis in vivo. However, data from these types of systems is most useful when the preservation of function, for example, between a human in vitro testing system and an intact human organ or tissue, is understood. Quantitative systems modeling provides methods to address the challenge of assessing preservation of mechanism across scales of biological complexity. The speakers in this session will tackle various aspects of the QST modeling and, in particular, the challenge of developing multi-scale and multidimensional quantitative systems models. The session will open with a summary of recent progress toward merging systems models with traditional PBPK models into complex multi-scale and multidimensional QST models. Following this introduction, the presenters will focus on case studies illustrating the potential for QST modeling to improve clinical to clinical safety by focusing on common organ targets for drug-induced injury, liver, kidney, gastrointestinal systems, and heart. The speakers will summarize progress in the field, illustrate new concepts that are nearing application, and highlight the opportunities and challenges in achieving a more quantitative estimate of human safety.

1168 Challenges and Opportunities in the Application of Integrated Multi-scale Systems Modeling

C. Fisher. Certara UK Limited, Sheffield, United Kingdom.

Fundamental to any QST model is an understanding of the relationship between drug exposure and critical biological response mechanisms underlying safety and pharmacology. As a result, the QST approach is multi-disciplinary in nature drawing on expertise from DMPK scientists, safety scientists, and computational modelers. Physiologically-based pharmacokinetic (PBPK) models integrate population specific data (systems parameters) and compound specific parameters to predict the absorption, distribution, metabolism and elimination of compounds in the body, and subsequently enable the translation of these processes across populations and preclinical species. Such models represent a branch of quantitative systems biology and can act as an integrating hub for models describing relevant safety pathways at the physiological, tissue, and translational level. PBPK simulation of tissue/tissue exposure profiles can provide the operating concentrations for tissue/cellular level QST models capturing mechanisms underlying cellular division, differentiation, death, repair, and core metabolism. These models can be further informed by omics data sets (e.g. transcriptomics, proteomics)
and metabolomics) and integrated models can range from the qualitative to the quantitative in their parameterization and simulation output. The ultimate goal is a multi-scale modelling framework spanning levels of biological organization and mechanistic resolution integrating multiple data sources. This will enable an iterative workflow of hypothesis formulation, model-based prediction, testing and model refinement operating in parallel to, and simultaneously informing, the discovery and development pipeline from target identification through to market. Sharing experiences and expertise from the IMI-TransQST project multi-disciplinary consortium, this talk will introduce the potential of such an approach, summarize the current state of the art, and remaining challenges ahead.

1169 Modeling Stress Responses across Scales of Complexity: From High Content Imaging in Cells to Modeling Co-expression Networks


Drug safety testing is a multi-scale process by design, often beginning in cell-based models before progressing to clinical and nonclinical studies. Quantitative systems toxicology models capture the multi-scale nature of drug toxicity from injury at the cellular level, to adverse outcome in tissues and organs. At inception of injury, cellular stress response pathways allow cells to adapt or with sustained activation lead to cell injury and signal progression to adverse cellular and tissue outcomes. Drug-induced liver injury (DILI) and drug-induced kidney injury (DIKI) remain important concern in drug development and an area where QST modeling can improve translational safety predictions. This presentation focuses on modeling drug-induced cellular stress response pathways in liver and kidney models across different scales of complexity. First, high content single live cell confocal imaging of GFP-tagged reporter proteins can elucidate dynamic stress-responsive transcription factors, e.g. Nrf2, Xbp1, p53 and NF-xB, and corresponding downstream target proteins, allowing detailed quantification of cellular stress-responsive and inflammatory pathways after drug treatments. The detailed temporal- and concentration-dependent dynamics of stress pathways are used to develop computational models of stress responses linking drug treatment to liver and kidney toxicity. Secondly, in vitro stress responses are linked to in vivo stress response pathways by building gene co-expression networks for both liver and kidney. Activation levels of the various stress gene networks by the above cellular stress responsive transcription factors are assessed systematically and quantitatively for both DILI and DIKI compounds. Recently, we have linked the stress response networks to the association of the various network perturbations that occur concurrent with adverse pathological outcomes in liver and kidney providing mechanistic insights into pathogenesis. Ultimately, mapping cellular stress response network activation and the preservation of network across scale of complexity from cells to tissues and across species will enable quantitative translational risk assessment for new drugs entering clinical trials.

1170 Modeling Cardiovascular Safety Using a Quantitative Systems Pharmacology Approach

D. Leishman. Lilly Research Laboratories, Indianapolis, IN.

Cardiac function and frank injury to the myocardium itself are among the primary causes of attrition in both clinical and nonclinical studies. Computational models of cardiac electrophysiology and hemodynamics have been around since the 60’s and 70’s, respectively. This presentation will describe the use of these mature models and their recent refinement over the past year to address critical questions in drug development. In illustrating the utility of QST models a test set of 55 drugs (32 torsadogenic drugs and 23 nortorsadogenic) were used to demonstrate the increased weight of evidence and improved negative and positive predictive values which can be achieved when using the same in vitro ion channel data as inputs to a QST model to derive integrated outputs prior to use of machine learning compared to the more traditional approach where the in vitro data themselves are used. The presentation will also illustrate that the QST models can predict continuous biomarkers, such as the electrocardiogram QTc interval, as well as toxicity classifications. In both cases the QST models can be combined with PBPK models to move beyond pharmacological properties and into patient-dependent intrinsic and extrinsic factors. The presentation will show how this was used to explore cardiac risk in the use of chloroquine and hydroxychloroquine in previously unused dosing regimens to treat covid-19. In addition to the constantly-developing cardiac electrophysiology model examples further case studies will involve examples, possible in only the last few months, related to cardiac contractility, blood pressure and heart rate.

1171 Multi-scale Modeling Approaches to Describe Drug-Induced Gut Toxicity

C. Pin. AstraZeneca, Cambridge, United Kingdom. Sponsor: J. Stevens

The gut is not only a primary route of drug absorption, but is a well-known target for anti-proliferative compounds and can be dose limiting in clinical studies. The relationship between exposure and disruption of gut homeostasis are poorly understood. The dynamic nature of gut biology including rapid cell turnover and unique immune functions make it a complex tissue with unique challenges. Multiscale models enable the prediction of toxicity and recovery at multiple spatial and temporal scales. They are instrumental to quantify how this organ responds to toxicological challenges at a whole and at each structural level. In this presentation, we will use recent data and models generated by the TransQST consortium to illustrate the application of analytical approaches and computational models, such as agent based models, to bridge scales and connect molecular toxicity with epithelial disruption and subsequent adverse effects. In this study we show how mental inputs are integrated and predictions generated at the molecular, cellular, tissue and clinical level in mouse intestine during exposure to 5-Flourouracil (5-FU) and subsequent recovery. The approaches describe the pharmacokinetics (PK) of 5-FU and of its main active metabolites as well as their impact on the epithelial following administration. The dynamical response to 5-FU is modelled at several scales and include activation and repression of molecular pathways which regulate cell cycle arrest and apoptosis, disruption and recovery of the crypt and villus architecture, changes in the epithelial barrier and risk of clinical adverse effects. We demonstrate that the model recapitulates observed changes over time gathered at the transcriptional, cellular, tissue and clinical level during 5-FU treatment. Further work to describe the integration in our models of inputs generated with novel preclinical technologies such as in vitro cultured GI organoids will also be discussed.

1172 Closing the Data Gap: Assessing Population Variability Using Next-Generation Tools in Toxicology

D. You. NIEHS/NTP, Research Triangle Park, NC.

A goal of chemical toxicity assessment is to derive a “safe” human dose that is protective for the majority of the human population. Despite decades of knowledge highlighting the importance of genetic sequence variation on toxicity outcomes, conventional toxicity testing uses only a limited number of donor cells or animal strains, neither of which appropriately account for interindividual variability inherent in a diverse human population. To address this data gap, regulators apply a default uncertainty factor to account for potential interindividual differences in toxicokinetic (TK) and toxicodynamic (TD) parameters and to provide a margin of safety. However, as suggested by prior studies, this default factor may not be sufficiently protective of sensitive subpopulations, depending on the chemical x gene interaction underlying sensitivity. Therefore, it is critical to consider population variability in toxicity testing to more accurately and comprehensively evaluate risks of xenobiotic exposure and identify adequately protective reference doses. Recent developments in in vitro and in silico models have greatly enhanced our ability to perform data-rich population-based toxicity testing. Such developments include cell-based population models composed of diverse individuals; advancements and increased throughput of molecular tools such as RNA-sequencing and high content imaging that can provide a great depth of information about population responses; and application of sophisticated statistical modeling to calculate the uncertainty around the population data and to provide more precise estimates of TK and TD variability. The goal of this session is to communicate the importance of assessing the population dynamics in hazard and risk assessment and to discuss emerging tools in toxicology that facilitate the advancement of population-based analysis. Four speakers from multiple disciplines and sectors will discuss the development and application of various population-based approaches in different areas of toxicology. The first two speakers will discuss statistical approaches and models to address population-based TK variability. The first speaker will talk about the development of the ILSI-human population-based TK modeling and its implementation in risk prioritization. The second speaker will introduce an open-source physiological based pharmacokinetic modeling tool that addresses the population variability specific to the pregnant population, a key sensitive subpopulation for whom toxicity testing is challenging and safety data are sparse. The speaker will also highlight how to assess the safety of xenobiotics during pregnancy and guide regulatory decisions to better protect perinatal health. The next two talks will demonstrate the utility of in vitro population-based assays for investigating TD variability. The third speaker will discuss the power of utilizing a human induced pluripotent stem cell derived population model and how coupling it to Bayesian concentration response modeling can provide robust and accurate data on the population variability in cardiotoxicity risk from chemical exposure. The last speaker will introduce a high content imaging- based in...
Physiologically based pharmacokinetic (PBPK) modeling is a useful computational tool that can incorporate both the drug-specific properties and time-varying gestational physiological changes to predict pregnancy-related PK changes. Moreover, PBPK models allow for the study of the effects of inter-individual variabilities on drug disposition. Recently, the dynamic changes in the variabilities of physiological parameters during pregnancy have been characterized which allowed for the development and extension of a deterministic pregnancy PBPK model for antipsychotics developed using the open-source programming language R to a probabilistic version representative of this life-stage. Aripiprazole, risperidone and clozapine were evaluated as our case studies due to their continued use by pregnant women and data availability. In vitro or in vivo extrapolation (IVIVE) of the several contributing hepatic enzyme activities and their gestational changes was applied to simulate the trimester-specific changes in drug metabolism over the course of pregnancy. This work was supported, in part, by grants from US EPA (STAR R0835612002) and NIH (T32 ES026568).

Human induced pluripotent stem cell (iPSC)-derived cardiomyocytes are an established model for testing potential chemical hazards. Inter-individual variability in toxicodynamic sensitivity has also been demonstrated in vitro; however, quantitative characterization of the population-wide variability has not been fully explored. We sought to develop a method to address this gap by combining a population-based iPSC-derived cardiomyocyte model with Bayesian concentration-response modeling. A total of 146 compounds, including 44 pharmaceuticals and 82 environmental chemicals, were tested in iPSC-derived cardiomyocytes from 43 non-diseased humans. Hierarchical Bayesian population concentration-response modeling was conducted for five phenotypes reflecting cardiomyocyte function or viability. Toxicodynamic variability was quantified through the derivation of chemical- and phenotype-specific variability factors (TDVF). Toxicokinetic modeling was used for probabilistic in vitro-to-in vivo extrapolation in order to derive population-wide margins of safety (MOS) for pharmaceuticals and margins of exposure (MOE) for environmental chemicals. Pharmaceuticals were found to be active across all phenotypes. Over half of tested environmental chemicals showed activity in at least one phenotype, most commonly positive chronotropy. TDVF estimates for the functional phenotypes were greater than those for cell viability, usually exceeding the generally-assumed default of 3. Population variability-based MOS for pharmaceuticals were correctly predicted to be relatively narrow (between 10-100); however, MOE for environmental chemicals, based on population exposure estimates, generally exceeded 1000, suggesting they pose little risk at general population exposures even to sensitive subpopulations. This study represents a first of its kind in vitro model that can be used to characterize toxicodynamic population variability in cardiotoxic risk. This work was supported, in part, by grants from US EPA (STAR R0835612002) and NIH (T32 ES026568).

Genetic sequence variation between individuals is recognized as a contributing factor for developmental neurotoxicity (DNT). Yet, there are no assays incorporating sufficient genetic diversity to capture genetic drivers of DNT susceptibility. DNT has been found in the general population. However, the genetic diversity is not captured using limited population-based methods. Population-wide margins of safety (MOS) for pharmaceuticals and margins of exposure (MOE) for environmental chemicals, based on population exposure estimates, are generally exceeded 1000, suggesting they pose little risk at general population exposures even to sensitive subpopulations. This study represents a first of its kind in vitro model that can be used to characterize toxicodynamic population variability in cardiotoxic risk. This work was supported, in part, by grants from US EPA (STAR R0835612002) and NIH (T32 ES026568).
1177 Hereditary Disorders of Manganese Metabolism: Mechanisms, Clinical Presentation, and Neurotoxicity

S. Mukhopadhyay. University of Texas at Austin, Austin, TX.

The last decade has seen a revolution in Mn neurobiology due to the discoveries of three hereditary disorders of Mn metabolism—mutations in the transporters SLC30A10 or SLC39A14 cause Mn neurotoxicity while mutations in the transporter SLC39A8 cause Mn deficiency. Work on these transporters and genetic disorders is transforming our understanding of Mn homeostasis, detoxification, and neurotoxicity. This session brings together the leading experts in the field and integrates human clinical studies (Dr. Gospe) with epidemiological (Dr. Broberg) and cutting-edge basic science studies (Drs. Mukhopadhyay, Guilarte, and Knutson) to present the very latest work in this area. Due to the timeliness of the topic, the Symposium will be of wide interest to neurotoxicologists, metal biologists, geneticists, epidemiologists, and clinicians.

1178 Understanding Manganese Excretion and Neurotoxicity by Studying Two Rare Genetic Diseases

S. Mukhopadhyay. University of Texas at Austin, Austin, TX.

Over the last few years, homozygous loss of function in SLC30A10 or SLC39A14 were reported to induce severe Mn neurotoxicity. Our recent studies revealed that SLC30A10 is a Mn efflux transporter, which, at the cellular level, transports Mn from the cytosol to the cell exterior and protects against Mn toxicity. Further, SLC39A14 primarily transports Mn into cells. Despite transporting Mn in opposite directions, at the whole organism level, these two transporters cooperatively mediate Mn excretion from full-body and tissue-specific Slc30a10 or Slc39a14 knockout mice, indicating that SLC39A14 transports Mn from blood into liver and intestines, and SLC30A10 then excretes the intracellular Mn into bile and feces. Furthermore, our recent findings revealed that brain Mn levels are primarily controlled by the excretory activity of these transporters in the digestive system. This talk will give an overview of current state of knowledge about the inherited disorders of Mn metabolism, by providing an overview of the changes seen in human patients, and highlighting similarities and differences observed in animal models. Subsequently, it will focus on the excretory function of SLC30A10 and SLC39A14, and discuss the unexpected finding that the primary mode of the homeostatic control of brain Mn is excretion via the digestive system. A major highlight will be to provide the mechanism of disease due to mutations in these transporters and relate these findings to manganese neurotoxicity evident in the general population. Additionally, in 2020, we discovered that Mn excretion is upregulated by a novel Hif1a-dependent signaling pathway that transcriptionally upregulates SLC30A10 in the liver during elevated Mn exposure. This talk will describe the mechanisms and consequences of this transcriptional response, and how it may be leveraged to find a treatment for Mn neurotoxicity.

1179 Natural History, Genetics, and Treatment of Hereditary Disorders of Manganese Homeostasis

S. Gospe. University of Washington, Seattle, Seattle, WA. Sponsor: S. Mukhopadhyay

Dr. Gospe will focus on the clinical presentation, diagnosis, and history of human patients suffering from hereditary disorders of manganese metabolism due to mutations in SLC30A10, SLC39A11 or SLC39A14. His lecture will describe how patients with biallelic pathogenic variants in SLC30A10 present with early-onset progressive extrapyramidal motor dysfunction together with polyneuropathy and progressive liver disease, while patients with biallelic pathogenic variants in SLC39A14 have disease isolated to the central nervous system. Both disorders are associated with marked hypermanganesemia together with central nervous system manganese deposition. In contrast, patients with biallelic pathogenic variants in SLC39A14 have low or undetectable blood manganese levels and present with infantile hypotonia, failure to thrive and intellectual disability. An important part of this lecture will be to review, compare, and contrast these three hereditary disorders of Mn metabolism—mutations in these three familial disorders of manganese homeostasis. Video examples of the effective treatments of patients affected by disorders of hypermanganesemia with either chelation or iron supplementation will be demonstrated. The clinical descriptions of these three hereditary disorders of manganese homeostasis provide the starting point for the animal- and cell-based studies that have been pursued over the past few years on these proteins. As such, this talk will complement other presentations in this symposium.

1180 Role of Manganese Transporter Genetics in the Susceptibility of Children to Environmental Manganese Exposure


The focus of this talk will be on our epidemiology studies that have led to the identification of widely-prevalent common single nucleotide polymorphisms in SLC30A10 and SLC39A8, present in ~20-30% of the population, that strongly modify biological Mn concentrations in humans. Further fine mapping studies of SLC30A10, SLC39A14 or SLC39A8 have identified novel polymorphisms with functional impact on Mn concentrations. By a Mendelian randomization study we have shown that Mn exposure early in life has negative effects on children’s neurodevelopment including neurobehavioral phenotypes, and we also found indications of impact on liver function. The polymorphisms in Mn transporters contribute to differences in sensitivity to Mn exposure from the environment, where girls that are genetically less efficient at Mn uptake may be at increased risk. We will also identify SLC39A8 as a possible therapeutic target for disorders of brain Mn accumulation. In addition to describing clinical findings in patients by Dr. Gospe, and the other presentations focused on the function of these transporters in cells and rodents.

1181 Is SLC39A9 Required for Brain Manganese Accumulation? Evidence from Studies Using Knockout Mice


This lecture will focus on our studies on the Mn importer, SLC39A9. Studies in S39a9d-inducible knockout (Slc39a9d KO) and hepatocyte-specific Slc39a8 KO mice have revealed that SLC39A9 plays an essential role in Mn homeostasis by functioning in the liver, where it relocates Mn from the bile. Accordingly, loss of SLC39A9 increases biliary Mn losses, resulting in Mn deficiency. In an effort to define extrahepatic functions of SLC39A9 in Mn homeostasis, we crossed S39a9d KO mice with Slc39a14 knockout (Slc39a14 KO) mice to generate double-knockout Slc39a9d KO;Slc39a14 KO animals. Slc39a14 KO mice display impaired hepatic uptake of Mn and impaired Mn excretion, resulting in hypermanganesemia and Mn accumulation in various tissues, most notably bone, brain, and kidney. We find that while Slc39a14 KO;Slc39a8d KO mice load similar amounts of Mn in the bone and kidney as do Slc39a14 KO mice, they show markedly lower concentrations of brain Mn, suggesting that SLC39A9 is required for brain Mn accumulation. Consistent with this hypothesis, immunofluorescence analysis of mouse brain sections indicates that SLC39A9 is abundantly expressed in the choroid plexus, a principal site of Mn entry into the brain when plasma Mn concentrations are elevated. Overall, these studies have begun to define extrahepatic roles of SLC39A9 in Mn homeostasis; they also identify SLC39A9 as a promising therapeutic target for disorders of brain Mn accumulation. In addition to describing the above data, this presentation will relate findings in our mouse model with clinical presentations seen in human patients suffering from Mn deficiency due to loss-of-function mutations in SLC39A9.

1182 SLC39A14 Knockout Mice: A Genetic Model to Study Manganese Neurotoxicity

T. Guilarte. Florida International University, Miami, FL.

SLC39A14 is now recognized as a Mn influx transporter. Loss of function mutations of SLC39A14 result in behavioral manifestations of Parkinsonism with dystonia and responsive to tetrabenazine (used to treat idiopathic Parkinson’s disease (PD)). The use of the SLC39A14 knockout (KO) mouse provides a powerful tool to study and describe the neuropathogenesis of chronic Mn exposure along the lifespan. Assessment of Mn concentrations in blood and striatum of postnatal day (PN) 60 young adult SLC39A14-KO male and female mice, indicates highly significant increase in Mn concentrations in both blood (18-20 fold) and striatum (5-6 fold) relative to WT. Behavioral characterization of PN60 SLC39A14-KO male and female mice showed significant locomotor deficits in distance travelled, vertical counts, and speed with a significant increase in resting time relative to WT. Analysis of striatal dopamine (DA) concentrations and its metabolites in PN60 male and female SLC39A14-KO mice show no differences in the concentrations of striatal DA, DOPAC, HVA, HVA/DOPAC, or HVA/DAs ratio in SLC39A14-KO mice relative to WT. Tyrosine hydroxylase (TH) immunohis-
to chemistry in the striatum of PN60 WT and KO male and female mice also showed no significant differences, supporting the lack of nigrostriatal dopaminergic neuron terminal degeneration in the presence of significant locomotor impairment. Finally, unbiased stereotaxic cell counting of TH-positive dopamine neurons in the substantia nigra pars compacta (SNpc) did not show any differences in DA neuron number of PN60 male or female SLC39A14-KO mice relative to WT. These findings are consistent with non-human primate studies from our laboratory indicating that Mn-induced locomotor deficits are not the result of degeneration of nigrostriatal DAergic neurons. We are currently pursuing studies on in vivo dopamine release in the striatum under basal and stimulated release conditions in male and female SLC39A14-KO and WT mice. Moreover, we are assessing the effect of elevated brain Mn concentrations in the SLC39A14-KO mice on cholinergic interneuron number in the striatum. Collectively, these results indicate that the neuropathological changes resulting from Mn overexposure are different as observed in IPD. Our results further suggest that other neuronal systems besides dopamine are involved in Mn-induced parkinsonism with dystonia.

### 1183 Are Aircraft Cabin Fume Releases a Cause for Toxicological Concern?

**A. Vale. University of Birmingham, Birmingham, United Kingdom.**

Studies that have analyzed the air of civilian and military aircraft have detected not only compounds traceable to various aviation fluids (jet fuel, lubricants, hydraulic fluid and coolants) but also other chemicals such as pyrethroids and ozone. On rare occasions, failure or over-filling of the oil reservoir may give rise to a “fume event” (visible smoke, haze, and/or odors), which has been estimated to occur on 0.05% of flights overall (IN 2,000). This Workshop will describe the features reported following fume events, critique the toxicological mechanisms proposed, and evaluate the investigations currently available to assess cabin crew and passengers. The largest investigation of cabin crew ever conducted (some 3,750 individuals) will be reported for the first time. This study, based on medical reports, showed that the majority of crew complained of odors similar to the smell of oil, “used socks,” or burned material. The symptoms most frequently experienced were headache, dizziness, and nausea (>20% each). The toxicological importance of various chemicals that air sampling has detected in cabin air will then be addressed. Some studies have identified tri-cresyl phosphate, an organophosphate used as a high-pressure lubricant in engine oil, in cabin air. Although the six ortho-isomers account for only 0.2% of all isomers, there is particular concern that tri-ortho-cresyl phosphate may be responsible for neurological sequelae, as this metabolite can bind both to human acetylcholinesterase (producing acute organophosphorus poisoning) and to Neuropathic Target Esterase (NTE), thereby initiating organophosphate-induced delayed neuropathy. High ozone concentrations, which are well documented on long-haul flights, can produce eye, nose, throat, and respiratory features. Pyrethroid spraying on some flights, which helps ameliorate airborne diseases, leads to clinically significant pyrethroid concentrations. Pyrethroids can also result in eye, nose, throat, and respiratory features. Finally, a critical assessment will be made as to whether analytical investigations can assist in the diagnosis of cabin fume events. An adduct of CBDP with butyrylcholinesterase was first reported to be present in the serum of a group of asymptomatic aircraft passengers in 2011. More recently, adducts of the cresyl-benzodioxa-phosphorin-oxide-derived phospholipid group with tyrosine residues have been detected, as well as histidine- and lysine-adducts with ortho-cresyl. Such peptides have been used as biomarkers in diverse smaller studies and case reports. However, these analytical methods are extremely sensitive and a correlation between these biomarkers and development of signs and symptoms has not yet been established.

### 1184 Symptoms Reported by Cabin Crew after a Cabin Fume Event 2009–2019

**N. Glaser, Bundesinstitut für Risikobewertung (BfR), Munich, Germany.**

Sponsor: A. Vale

Between 2009 and 2019, more than 1,400 cabin fume events involving some 3,750 cabin crew were reported to the German Federal Institute for Risk Assessment as required by law (Chemikalienverordnung § 16c). Women (61%) were involved more frequently than men (36%) and more flight attendants (66%) reported exposures than pilots (19%). The majority (77%) of the individuals complained of odors, mainly similar to the smell of oil, “used socks” or burned material. The symptoms most frequently experienced were headache, dizziness, and nausea (>20% each). A smaller percentage of the patients reported “prickly sensations” (paresthesiae) and numbness (<10%, each). Eighteen per cent of individuals did not report any symptoms (WHO/IPCS/EC/EA/PCCT Poisonsing Severity Score (PSS) of 0), 69% had a PSS of 1 (minor symptoms) and 4% had a PSS of 2 (moderate symptoms). No severe symptoms (PSS 3) were reported. Patients with PSS 2 showed most commonly hypertension (cause not given) and syncope/fainting or prolonged symptoms such as headaches, weakness or cognitive impairment. Laboratory results such as cholinesterase activity, oxygen saturation, carboxyhemoglobin and methemoglobin concentrations, did not show abnormal values indicating poisoning. In conclusion, symptoms reported were in general non-specific and variable between patients. Due to this variability and lack of biomonitoring data it was not possible to match symptoms with potential toxic agents.

### 1185 Is There a Toxicalogical Explanation for the Clinical Features Reported following Cabin Fume Events?

**A. Vale. University of Birmingham, Birmingham, United Kingdom.**

Some studies that have analyzes the air of civilian and military aircraft have detected tri-cresyl phosphate, an organophosphate, used as a high pressure lubricant in engine oil. The six ortho-isomers account for only 0.2% of all isomers: two are di-ortho isomers and one is the tri-ortho isomer (tri-ortho-cresyl phosphate), which is bioactivated by CYP 1A2 and 3A4 to 2-(ortho-cresyl)-4H-1,2,3-benzodioxaphosphorin-2-one (CBDP). This metabolite can bind to human acetylcholinesterase and Neuropathic Target Esterase (NTE). As a result, exposure to substantial amounts of tri-ortho-cresyl phosphate (by ingestion not inhalation) has led to features of acute organophosphorus poisoning due to inhibition of acetylcholinesterase and Neuropathic Target Esterase (NTE). However, no analytically confirmed cases of acute organophosphorus poisoning or organophosphate-induced delayed neuropathy have been reported. Ozone enters the cabin from outside the aircraft, particularly during intercontinental polar routes, and during late winter/early spring. When first installed ozone catalytic converters decompose 90-98% of the ozone present, but converters lose their efficiency because of other cabin contaminants (“surface poisoning”). High ozone concentrations can produce eye, ENT, and respiratory features, such as dry mouth or lips, dry and itchy eyes, nasal stuffiness. Pyrethroid spraying (pre-flight, immediately before take-off, and at the top of descent on some flights) to help ameliorate airborne diseases leads to clinically significant pyrethroid concentrations, which can produce nasal irritation, throat irritation, sneezing, cough, dizziness, headache, and nausea.

### 1186 Can Analytical Investigations Assist in the Diagnosis of Cabin Fume Events?

**H. Thiermann, Bundeswehr Institute of Pharmacology and Toxicology, Munich, Germany.**

Tri-ortho-cresyl phosphate has been suggested as a component that might be present following cabin fume events. Although possible toxic mechanisms might be mediated by binding to acetylcholinesterase or neuropathy target esterase, only high level inhibition of these enzymes is associated with clinical symptoms. Whether possibly further chemicals, such as pyrethroids or ozone. On rare occasions, failure or over-filling of the oil reservoir may give rise to a “fume event” (visible smoke, haze, and/or odors), thereby initiating organophosphate-induced delayed neuropathy. High ozone concentrations, which are well documented on long-haul flights, can produce eye, nose, throat, and respiratory features. Pyrethroid spraying on some flights, which helps ameliorate airborne diseases, leads to clinically significant pyrethroid concentrations, which can produce nasal irritation, throat irritation, sneezing, cough, dizziness, headache, and nausea.

**Virtual 2021 SOT Annual Meeting and ToxExpo**
This timely and interactive session will specifically address the issue of how to maintain health and wellness while navigating through the stress of graduate school and early careers. Over the last several years, mental stress has become an overwhelming situation, particularly for certain generations. Seventy-six percent of millennials report that the biggest cause for stress is work related. In addition, 44% of both Millennials (1981-1996) and Gen Xers (1965-1980) report experiencing irritability or anger due to stress, compared with 36% of Baby Boomers (1946-1964) and 15% of the Silent Generation (1928-1945). While the common stressors throughout the generations remain fairly constant, this presentation will discuss certain factors that could contribute to these generational mental health differences, including whether younger generations are more open about expressing their stress and the role of 24/7 connectivity and social media play. This session, designed for all generations, will cover issues such as (1) what factors and determinants contribute to the current sense of being “overwhelmed”; (2) how we can adapt to achieve the wellness we all need to survive and be successful both in life and our career; (3) how the COVID pandemic has impacted research productivity and creativity; and (4) in a post-COVID world, how graduate education will change and how we can maintain our “cool.” Following the presentations (each 15-20 minutes), the session participants will form medium-sized groups and provide one to two points each regarding the following topics: (1) What contributes to our professional stress level, and what are some potential coping strategies we can use to deal with it? (2) How do we see education/career development changing, and how do we maintain our health and wellness in a post-COVID world? The speakers will help guide the breakout group discussions and collect discussion points to report back for a final panel discussion with audience participation. To ensure a diverse perspective, we will recruit approximately 10 SOF members representing our diverse membership (i.e., gender, race, ethnicity, LGBT+, career stage, and sector) to facilitate the breakout group discussions. Participants will benefit from the opportunity to engage in a constructive dialogue regarding such issues as coping tools for handling mental stress during these difficult years, how to be the mentor/supervisor your trainee needs, and the art of communication.
The negative mental health impacts of inequity are well documented. During the graduate student to early career stages, the intensity and exhaustive work often deemed as a requirement for success is multiplied with the realities of inequity in academic spaces and in daily lives. Social media has been a space for academic professionals to connect with others along shared identity and commonly lived experiences. These spaces are reported to be valuable for sharing professional work and, if one so chooses, personal aspects of one’s life – sometimes including experiences with inequity. While benefits of finding and sustaining community are also documented, finding community on social media represents a duality that often exposes social media users to positive experiences and opportunities to contribute to equity work while also exacerbating the challenges they face. This talk will discuss models of social media community, mental health benefits and harms of engagement through social media platforms, and the cost of being “plugged in” for long periods of time, which the COVID pandemic has magnified.

Tips for Managing Stress as an Early Career Scientist
L. Lewis. Takeda Pharmaceutical Company Limited, Medford, MA.

Transitioning from academia into industry can be both exciting and challenging. This talk will focus on how to manage stress while making the switch from being a graduate student or postdoctoral fellow to working as an early career scientist in industry. Defending, interviewing for jobs and finishing up research in academic labs are examples of events that may induce mental and physical stress on individuals. In addition, young professionals encounter stress when adjusting to their new work environment and job duties. As an early career scientist in industry, individuals may need to adapt to tight project timelines and a more business-oriented approach towards research. Within this talk, strategies to cope with stress as an early career scientist will be shared as well as perspectives of how the COVID pandemic as impacted “business as normal and the ability to transition into career opportunities”.

New Approach Methods for Cancer Risk Assessment

New approach methods (NAMs) are rapidly emerging as cutting-edge tools to modernize cancer risk assessment. Such tools offer the promise of mechanistic insight to support structured frameworks for regulatory decision-making. Traditional apical endpoints, such as those measured in the rodent cancer bioassays are used to identify potential human carcinogens. Rodent models (e.g. carcinogenic bioassays, food additives, and pharmaceuticals; however, decades of research have underscored limitations in the rodent cancer bioassays. These limitations provide opportunities to develop and refine NAMs for targeted use to support cancer risk assessment. Experts are working to modernize carcinogenicity testing with mechanistic approaches that reduce testing on animals and may provide more human health-protective information. This session has been modified from a previously accepted 2020 SOT Annual Meeting proposal to focus regulatory, industry, and nongovernmental organizations (NGOs) expert discussion toward current opportunities and lessons learned in the development and utilization of NAMs for human-relevant cancer risk and safety assessment. Speakers will present timely research in NAMs, including the ongoing Health Effects Innovation Initiative, which is working toward modernizing its carcinogenicity testing program, as well as a presentation highlighting the use of gene expression endpoints in short-term animal studies to identify potential human carcinogens. This talk will include lessons learned while developing NAMs for carcinogenicity assessment, as well as practical considerations for use of NAMs for regulatory decision-making. The discussion panel will be composed of Workshop speakers and session Chairs, representing experts from government, academic, industry, and NGOs. Overall, this Workshop will be interesting to a wide range of stakeholders, including regulators, cancer toxicologists, systems modelers, and industry scientists.

Development of an Adverse Outcome Pathway Network to Support an Integrated Approach to Testing and Assessment of Carcinogenic Risk
S. Stafford. Lhasa Limited, Leeds, United Kingdom. Sponsor: N. Kleinsteuber

The prediction of human carcinogenicity and related toxicity endpoints has always been a principal area of concern during chemical safety assessments. Historically, the two-year rodent bioassay has been used as the ultimate method for assessing potential human carcinogenic risk. More recently, new approach methods (NAMs) have been developed to predict carcinogenic risk, including both in vitro and in silico techniques. However, while there is some guidance on how to integrate specific pieces of evidence in well-defined use cases, a unified approach to combination of data for risk assessment has yet to emerge. One approach to creating a framework of NAMs for regulatory application is to utilize the concept of adverse outcome pathways (AOPs). This presentation will highlight the use of an expert rule-based SAR system, Derek
Nexus, in the development of a carcinogenicity AOP network containing over 35 molecular initiating events (MIEs) which link to the adverse outcome of carcinogenicity via more than 350 pathways. This network can be used in combination with in silico models and in vitro assays as a profiling tool for carcinogenicity mode of action prediction. Different types of information can be applied to give a transparent, interpretable and robust prediction of carcinogenic potential. The repository of knowledge and data that has been developed gives more detailed information on potential pathways and allows for the integration of existing and emerging in silico, *in vitro* and *in vivo* test results at the protein level to support an overall conclusion on carcinogenic potential.

**W 1199 Toward Replacing the Two-Year Bioassay with Short-Term NAMs: Genomic and Nongenomic Thresholds Can Identify Rat Liver Tumorigens**

C. Corton, US EPA/ORD, Research Triangle Park, NC.

Traditional data sources for cancer hazard assessment are resource-intensive, retrospective, and not feasible for the vast majority of environmental chemicals. Incorporation of quantitative genomic and nongenomic data from short-term rodent studies may adequately define protective thresholds for potential tumorigens as a bridge to move from current testing to greater reliance on *in vitro* assays. We hypothesized that gene expression biomarkers that measure the activity of potential mutagens (e.g., rodent liver cancer adverse outcome pathways as well as liver weight to body weight (LW/BW) and clinical chemistry (ClinChem) endpoints exhibit chemical-independent thresholds beyond which cancer occurs, and the thresholds could be used together as a NAM to predict liver cancer. The hypothesis was tested by defining thresholds of gene expression biomarkers of liver cancer from using training datasets from the 77 and 86 chemicals in the TG-GATES and DrugMatrix datasets, respectively and testing them in a number of contexts. The biomarker tests consisting of 7-113 genes included those that predict genotoxicity, cytotoxicity, and activation of Ahr, Car, ER, or PPARs. Thresholds were calculated as the maximum values derived from exposures that do not lead to liver cancer. In all cases, clear threshold values could be identified that were consistent across training and test sets. Thresholds derived from the TG-GATES study were not very predictive of liver tumorigens in the DrugMatrix study (77-81%). In contrast, thresholds derived from the DrugMatrix study were predictive in the TG-GATES study (84-100%). The DrugMatrix-derived thresholds were most predictive when applied to test sets of 7d and 14d treatments (100% and 99%, respectively). In addition, thresholds derived from just 12 genes (2/biomarker) as well as LW/BW and ClinChem endpoints exhibited high predictive accuracy (up to 94%). These findings support the idea that genomic and nongenomic changes measured after short-term exposures can be used to establish threshold estimates or “molecular tipping points” that are predictive of later-life outcomes. This abstract does not reflect US EPA policy.

**W 1200 Using Preclinical Models to Understand Metastasis**

K. Tanner, National Cancer Institute, Rockville, MD. Sponsor: N. Kleinsteuber

Much of our knowledge on tumor progression and metastasis has been obtained using murine models where dissecting the role of the physical microenvironment in regulating organotropism remain technically challenging. The zebrafish is rapidly becoming a model for studying tumor behavior at different stages of the metastatic cascade. Moreover, organs that are frequent sites of metastasis in human patients are well conserved between mammals and zebrafish. Our previous work has quantified tissue mechanics, and architecture of diverse organs to assess direct comparisons between zebrafish and mammalian systems. We recently developed a zebrafish model of human tumor metastasis and human macrophage migration and demonstrated that it recapitulates patterns of metastasis and tumor interactions. We discovered that human breast cancer cells that home to specific organs in mice also show non-random extravasation in an organ-specific manner within zebrafish. Specifically, cells that home to the murine brain also home to the zebrafish brain, whereas cells that home to the murine bone marrow also home to the embryonic bone marrow niche in larval fish. Extravasation was mediated by β1 integrin, where knockdown of 61 integrin reduced extravasation for both clones in the CVP but did not affect extravasation of brain-tropic clones in the brain. In contrast, silencing unconventional myosin 1b (MYO1B) redirected brain tropic clones from the zebrafish brain to the zebrafish CVP. We next asked if genes discovered using the zebrafish platform will also predict behavior in mice. We confirm that silencing of MYO1B using Crispr/cas 9 in human cells also reduced brain colonization in mice concomitantly with an increase in homing to the bone marrow niche. These data suggest that the zebrafish can be used as a rapid platform to assess key determinants of organ specific extravasation, which have potential utility to increase efficiency in chemical safety assessment.

**W 1201 The Need for Protocol Harmonization in the Advancement of Zebrafish as a Model for Toxicological Screening: Global Perspectives and Recent Advancements**

M. Behl, NIEHS, Research Triangle Park, NC.

Over the past decade, there has been much advancement in the use of zebrafish as a powerful alternative model in drug and toxicity screening. However, harmonization among protocols and methods used in zebrafish research is currently lacking, thereby resulting in divergent outcomes while testing the same set of compounds. Although there has been global consensus on an urgent need for protocol harmonization, this is the first time that a concerted effort has been made globally to evaluate protocols in a systematic way to understand underlying differences in outcomes. This Workshop highlights advancements from two major global efforts: (1) an effort led by the Organisation for Economic Co-operation and Development (OECD) on harmonization of protocol parameters in the field of developmental neurotoxicity and (2) a task force within the European Teratology Society (ETS) that was created for exploring the Zebrafish Embryo Developmental Toxicity Assay (ZEDTA) as an alternative for developmental toxicity testing. Following regulations in Europe with regard to use of nonmammalian models, and the recent directive issued by the US EPA to reduce funding for mammalian testing by 30% by 2025 and to eliminate all mammal testing by 2035, the use of complementary models such as zebrafish are expected to be on the rise. Hence, it is critical to understand how underlying study parameters may influence results as we move zebrafish forward for prioritization and prediction of toxicity outcomes. The Chair will provide a brief introduction on the need and timeliness of this Workshop to advance zebrafish research globally. The first speaker will shed light on recent advancements in the field with respect to toxicity screening and drug development, highlight needs and data gaps, and provide information on several global efforts to address these issues. The second speaker will provide an update on an OECD-initiated global harmonization project for developmental neurotoxicity (DNT) testing that is being conducted by an OECD Zebrafish DNT sub-group. This talk will highlight extensive discussion and protocol optimization from zebrafish experts globally. The third speaker will shed light on parallel efforts with respect to developmental toxicity harmonization that is being conducted as part of European (ETS) and US expert groups. The fourth speaker will discuss the state of the field with regard to uptake and metabolism in zebrafish and some caution that needs to be exercised during compound evaluation. Finally, the last speaker will provide case examples of how differences in protocols and analysis may impact outcomes and will shed light on a suggested path forward for protocol optimization as the zebrafish is being used more extensively as a complementary screening tool.

**W 1202 The Evolution of Zebrafish for Toxicology Research: State of the Field**

R. L. Tanguay, Oregon State University, Corvallis, OR.

Zebrafish is an established human relevant biomedical research model globally adopted across the entire translational spectrum. The use of the model for toxicological research exponentially expanded globally in the past decade to assess an ever-growing category of xenobiotics and real-world mixtures at a fraction of the costs of traditional hazard assessment. The Toxicogenomics Initiative (TGI) and DrugMatrix study provide a comprehensive data set of 163 chemicals, yielding results as we move zebrafish forward for prioritization and prediction of toxicity outcomes. The Chair will provide a brief introduction to further testing are different from studies aimed at conducting structure-activity relationships. Studies aimed at identifying the biological targets of exposure chemicals may differ from studies aimed at understanding the impacts of remediation on the biological activity of chemical mixtures. These different purposes lead to a number of important assay nuances that hamper direct data comparisons between laboratories. In the past decades, we have assessed thousands of individual chemicals, mixture and nanomaterials and have modified our screening platforms to increase throughput. Over time, deliberate changes in protocols, instrumentation and workflow has helped to identify key variables that influence experimental outcomes. For example, adding behavioral measure is key to increase sensitivity of the model.
Although the evolution of the zebrafish model for chemicals screening continues, this presentation will discuss several case studies illustrating the impact of study design on data outcome.

1203 An Inter-laboratory Case Study to Determine the Added Value of the Zebrafish Light-Dark Transition Test to Predict Developmental Neurotoxicity: Report from OECD DNT Expert Group

E. Hessel. Rijksinstituut voor Volksgezondheid en Milieu (RIVM), Utrecht, Netherlands. Sponsor: M. Behl

Developmental neurotoxicity (DNT) entails one of the most complex areas in toxicology. Development of the central nervous system is a complex process involving many different events within strictly controlled time frames and therefore might create different windows of vulnerability to chemical exposure. OECD test guidelines for DNT (TG 426 and 443) are only occasionally carried out and the predictivity of these in vivo animal tests for human effects may be limited. There is a high need for human-relevant in vitro models to assess DNT potential of chemicals. OECD is therefore building a guidance document containing a testing strategy to predict DNT. This testing strategy consists of a combination of in vitro tests encompassing the critical processes in brain development. Essential in this major effort is to have reliable protocols that mimic parts of neural development, preferably performed within a short time period. The aim of this study is to investigate the added value of the zebrafish model in this testing strategy. Zebrafish are until 120 hours post fertilization (hpf) not considered as experimental animals under the current European animal directive (2010/63/EU). The neurological system, the different neuron types and neurotransmitters are well studied and well conserved between zebrafish and other species including humans. The advantage of the zebrafish model in comparison to other in vitro assays is that whole brain development occurs within a relative short period of time and effects on chemicals on brain development and behaviour can be tested. A group of experts agreed on a protocol for the light-dark transition test to predict DNT. At 120 hpf, zebrafish are tested in the light-dark transition test after chemical exposure from 6-120 hpf in a 96 well plate. 7 concentrations and 12 larvae per concentration. 35 known DNT compounds will be tested in five different laboratories. Chlorpyrifos was selected as a positive control known to affect activity of the larvae in the dark period. First there is an acclimatisation period 5 minutes light on and 5 minutes light off. Thereafter, recording starts with 10 min on and 10 min off and another 10 min on and 10 min off. Some labs will test an additional 40 min on- 40 min off period after 40 min acclimatisation in the dark. Data-analysis will focus on locomotor activity (distance moved) of the larvae during the testing period. Benchmark dose analysis will be performed to determine the critical effect dose of each compound and for comparison of results across laboratories. Based on the results of this inter-laboratory case study the robustness, biological domain, and added of the light dark-transition assay to predict DNT will be discussed and determined. Future experiments will test more compounds in the light-dark transition tests and the added value of other zebrafish DNT behavioural test results will be discussed. The future goal is to add the zebrafish DNT assays to the OECD guidance document and to use this model to predict DNT.

1204 Advancements on Global Efforts for Protocol Harmonization for Zebrafish Toxicology Testing

A. Muriana. BBD BioPhenix, San Sebastian, Spain.

This presentation highlights efforts by a task force created within the European Teratology Society to evaluate Zebrafish Embryonic Developmental Toxicity Assay (ZEDTA) as an alternative for developmental toxicity testing. The goal of the ZEDTA task force is to harmonize the zebrafish embryonic assay for developmental toxicity. Given the upcoming ICH S5 revision (r3) the task force is proactively working toward harmonizing the zebrafish embryonic model to address issues of comparability and availability. As high sensitivity of the assay is key for regulatory acceptance, a literature review was performed to identify compounds that gave false-negative results in the ZEDTA. In total 27 out of 103 mammalian teratogens were misclassified in the ZEDTA by one or more labs. Study design, calculation of the teratogenic index, compound uptake, inconclusive maternal or animal data, lack of biotransformation, and inter-species differences in mode-of-action were identified as potential factors contributing to the misclassification of 20 compounds. Interestingly, the remaining false-negative compounds showed skeletal malformations in the in vivo rodent and/or non-rodent embryo fetal development studies. Therefore, a study was designed to assess whether an extension of the mode-of-action parameters and/or evaluation period in the ZEDTA after exposure to mammalian skeletal teratogens during organogenesis increases the concordance of the ZEDTA with the mammalian in vivo developmental toxicity studies. This talk summarizes latest outcomes by the ZEDTA Task force.

1205 Evaluation of the Uptake and Metabolism of Novel Toxicants by Zebrafish Larvae

L. D. Ellis. Health Canada, Ottawa, ON, Canada. Sponsor: M. Behl

Zebrafish larvae have classically been used as a high throughput model with which to test both the bio-activity and toxicity of known and novel compounds. Due to their small size, a single larvae can be housed in a single well of multi-well plates during their initial 5-7 days of development. The plates used can contain up to 96 wells with the volumes required per well as low as 10 microliters. Additionally whole larvae can be used allowing the concentrations tested and concentration-response patterns are assessed based on the bath concentrations of the compounds. In general, these models assume the “dialysis-bag” model, where the concentration of a compound in the larvae is the same as in the bath. However, previous work from our lab tested the levels of neuroactive compounds in larvae through LC-HRMS analysis and revealed that compounds can bio-accumulate in larvae to levels much higher than in the bath. Interestingly, the concentrations of the compounds found in the larvae did not always correlate in a linear fashion to the bath concentrations, which shifted the concentration-response profiles. We have now expanded on this initial study by testing the uptake of 10 known toxicants at 6, 8, 24, 72, and 120 hours post-fertilization to match the timelines of the fish embryo toxicity testing. Interestingly, comparisons between two of the compounds tested, Bisphenol A and Bisphenol S, revealed that based on bath concentrations the EC20 value of Bisphenol S is roughly 18 times larger than that of Bisphenol S. However, these large differences, the dose found in the larvae were determined to be very similar. It then appears that the large difference in EC20 values calculated from the bath concentrations is related to the uptake of each molecule rather than the actual toxicity of each chemical. In addition to the assessment of uptake, the analysis was able to show the metabolism of the compounds by the larvae. This is an important attribute of the study as compound metabolism may inactivate the compounds or create a bioactive metabolite. The profiling of the uptake of novel compounds by zebrafish larvae appears to be useful in order to better understand the toxicity profiles of novel compounds and may allow for more direct comparisons to mammalian studies.

1206 Understanding the Influence of Protocol Parameters in Zebrafish Embryonic Developmental and Neurotoxicity Screenings on Compound Toxicity Outcome Interpretation

J. Hsieh. NIEHS/NTP, Research Triangle Park, NC. Sponsor: M. Behl

Compound toxicity data obtained from independent zebrafish laboratories can vary, complicating the use of zebrafish screens for regulatory decisions. Possible reasons include the difference in assay design such as protocol parameters and methods for data analysis. We investigated this issue by utilizing data from NTP DNT-DIVER (https://sandbox.ntp.niehs.nih.gov/neutralotx/), which consists of data from zebrafish developmental toxicity (DT) and neurotoxicity (NT) screenings from three independent laboratories who tested the same set of 90-compounds provided by the NTP. The data were analyzed using the recently published approach to zebrafish data analysis based on benchmark concentration (BMC) modeling (Hsieh JH 2019). We compared the BMC results from three laboratories in three toxicity outcome categories: mortality, DT, and NT. The binary call (active/inactive) concordance between pairs of labs ranges from 62% to 88%, where the highest concordance was observed for DT data. By using the linear mixed effects models (LMM) with BMC as the outcome variable with compound as the random effects and data source (i.e., lab) as the fixed effects, we found that the BMC results are significantly different between labs in DT and NT data but not in mortality data. The difference in BMC values can be on average of up to 4-fold in DT data and up to 6-fold in NT data. By applying protocol parameters as the fixed effects to the LMM models, we identified and ranked protocol parameters based on their contributions in the amount of change of BMC values. By this approach, we observed that a decrease of potency may be related to the combined effect of several parameters, which include static dosing and embryo de-chiorionation for DT and embryo de-chiorination, earlier exposure, smaller exposure volume, and shorter testing time for NT. This talk will shed light on the need for further experiments with systematic controls so that the individual and combined effect of each parameter can be fully elucidated. Lessons learned and underlying principles are currently being applied to large global projects.
such as the OECD-led DNT project and the National Toxicology Program’s Systematic Evaluation on the Application of Zebrafish in Toxicology (SEAZIT) effort.

**1207 Revising Biology: Alternative Splicing in Toxicology**

M. Banerjee, University of Louisville, Louisville, KY.

Most eukaryotic genes consist of protein coding modules (exons) interspersed with stretches of non-coding modules (introns). During RNA maturation, the introns are excised out and the exons are joined by a precisely tuned mechanism called splicing. The modular nature of exons and introns makes it possible to generate several unique exon combinations from a single gene by a process called alternative splicing. Thus, a genome-wide alternative splicing program is a tightly coordinated process by which several mRNA and, consequently, protein isoforms can be generated from a single gene, often with different and sometimes completely opposing biological functions. About 90% of all human genes undergo alternative splicing, making it a major driver of gene regulation and generation of proteomic diversity. Recent studies unequivocally demonstrate that dysregulated alternative splicing is a common event upon a diverse range of toxic exposures. Data suggest that such dysregulated alternative splicing plays key roles in the pathogenesis of several diseases upon toxic exposures, especially cancer and immune responses. The goal of this session is to examine how alternative splicing modulates key physiological and pharmacological processes leading to disease outcomes/susceptibility because of toxic exposures. It will bring together a panel of experts to discuss and dissect the mechanisms by which dysregulated altered splicing brings about disease outcomes as well as modulates the effects of therapeutics intervention. The session will address pressing issues such as (1) How can alternative splicing regulate metabolism/detoxification/biotransformation of endogenous and exogenous toxicants and therapeutic molecules? (2) What are the mechanisms by which alternative splicing can modulate carcinogenesis? (3) What is the role of metals in bringing about genome-wide differential alternative splicing events leading to cancer? (4) What are the computational and experimental methods available for detecting and studying genome-wide alternative splicing? The first talk will address how alternative splicing in cytochrome P450 proteins can modulate toxicant metabolism upon environmental exposure leading to interindividual differences in health outcomes. The second talk will describe the role of splice switch in ARNT isoforms by exogenous and endogenous toxicants in autoimmune disorders and its potential as a putative therapeutic target. The third talk will explore the possible contributions of differential alternative splicing in the pathogenesis of chronic arsenic exposure-induced skin cancer. Following this session, the attendees will develop a clear understanding of the mechanistic role played by alternative splicing dysregulation as harbingers of a diseases state in studying genome-wide alternative splicing. The imbalance in isoform species can result in pathogenic changes in critical physiological immunomodulatory roles. Consequently, interference of normal AhR signaling by environmental toxicants influences the development of autoimmune disorders. However, little is known about the role of the AhR binding partner, aryl hydrocarbon receptor nuclear translocator (ARNT), in AhR immune signaling. ARNT is often described in toxicant induced AhR signaling as a constitutively expressed, non-regulated partner for AhR. However, our data challenge this assumption and show that the actual regulatory paradigm is more intricate. For instance, ARNT is expressed as two isoforms, isoform 1 and 3, which differ in only 15 amino acids present in isoform 1. Our studies indicate that the RNA-binding protein RBFOX2 controls the splicing switch of ARNT. We identified RBFOX2 binding motifs in ARNT pre-mRNA that controls the ratio of ARNT isoform 1 and 3 via alternative splicing. Moreover, different T cell polarization states lead to different levels of RBFOX2 expression and a concomitant change in ARNT isoform ratios. Despite their sequence similarity, we have found that the ARNT isoforms have opposing functions. Specifically, AhR activation by exogenous (e.g., TCDD or BP) or endogenous (e.g., L-kynurenine or FICZ) ligands induces phosphorylation of ARNT isoform 1 by CK2, which releases ARNT isoform 3 from E-box elements to bind AhR and promote optimal activity. Thus, RBFOX2-controlled splicing of ARNT after T cell activation leads to specific ARNT isoform ratios that modulate AhR signaling in a manner consistent with a particular polarized state. These data reveal new mechanisms by which chemical exposure may trigger immunotoxicity and suggest the possibility of immunomodulation by targeting ARNT splicing using splice switching oligonucleotides.

**1209 Proper T Cell Activation Requires Specific ARNT Alternative Splicing Patterns for Fine-Tuning AhR Signaling**

C. W. Wright, University of Texas Medical Branch at Galveston, Galveston, TX.

The AhR is a crucial regulator of normal T cell differentiation and other physiological immunomodulatory roles. Consequently, interference of normal AhR signaling by environmental toxicants influences the development of autoimmune disorders. However, little is known about the role of the AhR binding partner, aryl hydrocarbon receptor nuclear translocator (ARNT), in AhR immune signaling. ARNT is often described in toxicant induced AhR signaling as a constitutively expressed, non-regulated partner for AhR. However, our data challenge this assumption and show that the actual regulatory paradigm is more intricate. For instance, ARNT is expressed as two isoforms, isoform 1 and 3, which differ in only 15 amino acids present in isoform 1. Our studies indicate that the RNA-binding protein RBFOX2 controls the splicing switch of ARNT. We identified RBFOX2 binding motifs in ARNT pre-mRNA that controls the ratio of ARNT isoform 1 and 3 via alternative splicing. Moreover, different T cell polarization states lead to different levels of RBFOX2 expression and a concomitant change in ARNT isoform ratios. Despite their sequence similarity, we have found that the ARNT isoforms have opposing functions. Specifically, AhR activation by exogenous (e.g., TCDD or BP) or endogenous (e.g., L-kynurenine or FICZ) ligands induces phosphorylation of ARNT isoform 1 by CK2, which releases ARNT isoform 3 from E-box elements to bind AhR and promote optimal activity. Thus, RBFOX2-controlled splicing of ARNT after T cell activation leads to specific ARNT isoform ratios that modulate AhR signaling in a manner consistent with a particular polarized state. These data reveal new mechanisms by which chemical exposure may trigger immunotoxicity and suggest the possibility of immunomodulation by targeting ARNT splicing using splice switching oligonucleotides.

**1208 Alternative Splicing in the Cytochrome P450 Superfamily as Biomarkers of Chemical Exposure and Environmental Disease**

A. J. Annalora, Oregon State University, Corvallis, OR.

Genotoxic metabolite formation mediated by cytochromes P450 (CYP) proteins such as CYP1B1, play a well-established role in driving carcinogenesis. DNA and other cellular nucleophiles also have well-studied sensitivities to reactive oxygen species (ROS). Less well understood is the role that cellular stress from xenobiotic exposure contributes to altering RNA expression, stability, and splicing. Mechanistically, alternative splicing is highly complex and not fully understood. However, transcript-damaging ROS, molecular disruption of spliceosome function by xenobiotics, direct toxic effects by metals on nucleic acid and protein function, and nuclear receptor (NR)-driven modulation of splice site usage may all contribute to personalized transcriptome remodeling. Thus, alternative splicing may be a biomarker for chemical exposures, disease diagnosis and drug development. Our recent meta-analyses of transcript variant expansion in human CYP and NR genes suggest alternative splicing is common in cancer cells, and that deep RNA sequencing is required to accurately detect splice variant biomarkers of xenobiotic exposure and disease. This session will address how cassette exon diversification patterns among CYP and NR genes, which manifest subfamily-specific alternative splicing programs, provide the genome with added plasticity and resilience to adapt to changing cellular conditions. New observations from our CYP splicing-related research will also be presented, including: 1) A structure-activity relationship for model xenobiotics (n-alkyl-methyleneoxybenzenes or MDBs) that cause alternative splicing in the CYP2B12 subfamily based on differentials in receptor binding, RNA splicing, protein expression and CYP metabolic capacity; 2) The role that genetic polymorphisms (CYP3A5*3) and RNA secondary structural motifs (guanine-quadruplex (G4) structures) play in modulating CYP gene expression; and 3) Examples of splice switching oligonucleotides (OSOs) development targeting renal CYP3A5 for therapeutic purposes. While additional research is required to fully elucidate the adaptive role for alternative splicing in endo-xenobiotic metabolism, transcriptome expansion is clearly an important marker of chemical exposure and disease. Furthermore, the finding that alternative splicing may drive chemical resilience and immunity is emerging as an important concept in toxicology.

**1210 Differential Alternative Splicing as a Mechanism for Chronic Arsenic Exposure-Induced Squamous Cell Carcinoma**

A. P. Cardoso, University of Louisville, Louisville, KY.

Chronic arsenic exposure in drinking water is associated with an increased risk of developing cancers and non-cancerous diseases. 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. We hypothesized that chronic arsenic exposure causes global dysregulation of normal alternative splicing profile and sets the cells in a cancerous trajectory. Multiple cultures of immortalized human keratinocytes (HaCaT), each with or 100 mM NaAsO2, were maintained for 28 weeks. RNA-Seq was performed in cells harvested at 7, 19 and 28 weeks with subsequent rMATS analysis to identify 5 kinds of differential splicing events (skipped exons, mutually exclusive exons, alternative 5’ splice site, alternative 3’ splice site and retained introns). At least 600 significantly different alternative splicing events at each tested time point were observed, comprising all the five main types of alternative splicing and occurring both in the ORF and the UTR of genes. Data demonstrates that most of the genes were represented uniquely at each time point, with very limited overlap between the different time points. The relative proportions of each subtype were also variable across the different time points. Gene ontology (GO) analysis on the differentially alternatively spliced genes at 7-weeks time point found enrichment in several splicing related pathways (83 unique genes). These results suggest that genome-wide differential alternative splicing events could in
past be responsible for the changing proteomic landscape with time in arsenic-induced carcinogenesis and highlight the complex and dynamic role of alternative splicing in cancer progression. Inherent issues with the bioinformatic platforms used for alternative splicing analysis coupled to the dearth of isomophic specific antibodies make corroboration of these predictions at the proteomic level challenging.

1211 The Future of Uncertainty Factors with In Vitro Studies Using Human Cells

A. Hayes, University of South Florida, Tampa, FL.

Safety or uncertainty factors (UFs) are used by regulatory agencies to account for perceived deficits in toxicology data using animals. Toxicology now has technological tools that, if used directly to or humans, might yield responses without tools such as microphysiological systems. “New approach methodologies” (NAMs) is a reference to any nonanimal technology, methodology, approach, or combination used to provide information on chemical hazard and risk assessment. NAMs, including in vitro technology methods using human cells, are available from simple cell cultures to 3D models of human skin, liver, and other organs to similar human organs-on-a-chip. Although the focus of this debate is on organs-on-chips technology, the principles and application can be applied to other types of NAMs. NAMs are challenging the traditional “norm” of regulatory risk assessment that has been in place for many years, including uncertainty or safety factors. Are these factors needed for testing when human cells are used? This important question will be debated in the Roundtable discussion, with the hope of bringing some guidance to the development of 21st-century risk assessment. The question proposed is, “Will safety or uncertainty factors still be needed when using human cells?” This debate brings together two outstanding scientists to discuss and debate the issue. Michael Dousson, PhD, who co-authored the original paper detailing uncertainty factors, and Lorna Ewart, PhD, who was a leader in adopting organ-chip technology within the pharmaceutical industry, are our two debaters. Following the debate, Dr. Dousson and Ewart will be joined by Drs. Suzanne C. Fitzpatrick, US FDA; Silvia Barros, Universidade de São Paulo, Brazil; and Brinda Mahadevan, Abbott, India, in a panel discussion. Dr. Hayes will moderate the 30-minute panel discussion.

1212 Applications of Novel High-Throughput Approaches for Mechanism-Based Chemical Safety Assessment

M. Leist, Universität Konstanz, Konstanz, Germany.

There is a need for quantitative systems toxicology to take advantage of human-relevant in vitro test systems in providing mechanism-based solutions for chemical safety assessment. This is essential since classical animal-based testing approaches have shown a very limited ability to predict adverse human health outcomes under environmental exposure conditions. Moreover, there is an enormous need to properly characterize chemicals in the environment or approaching the market that humans may be exposed to that lack thorough safety evaluation. Efficient high-throughput methods, integrating mechanistic information based on the bioactivity of chemicals, represent a valid alternative for cost- and time-effective assessments of the possible biological consequences of exposure to environmental chemicals. These methods involve both high-throughput phenotypic screening that generates quantitative information at the single cell level as well as high-throughput transcriptomics that yields concentration-response information on gene expression changes at the cell population level. This session will demonstrate the use of transcriptomics-, metabolomics-, and proteomics-based approaches for characterization and/or predictions of mechanisms of action for potential use in chemical safety assessment. First, the use of high-throughput transcriptomics for identifying molecular biomarkers from short-term in vivo studies that forecast pathological manifestations in longer-term studies will be discussed. Second, the variability in sensitivity toward the activation of toxicity pathways in the human population will be discussed. This approach is based on high throughput transcriptomics-based concentration-response statistical modeling of 50 different primary hepatocyte human donors. In this context, the relevance in the application of setting safety factors also will be debated. Further, an approach for metabolomics-based read-across will be presented. Data extracted by a large (>1,000 compounds) metabolomics database (MetaMap®Tox) have been used to assess biological similarity among compounds. Additionally, the application of a refined in vitro teratology method that combines functional and morphological endpoints (e.g., neuronal rosettes formation) with mechanistic endpoints (e.g., transcriptome changes) for efficient screening of developmental toxicants will be discussed. Finally, the use of a high-content imaging-based pheno typings profiling method known as “Cell Painting” for bioactivity screening of chemicals, potency estimation, and prediction of putative mechanisms of action will be presented. Participants in this session will gain a broader understanding of how high-throughput screening approaches for assessing chemical effects on the transcriptome, metabolome, and phenotype can be used for mechanism-based chemical safety assessment.

1213 Early Prediction of Late Adverse Outcome Using Benchmark Dose Modeling of High-Throughput Transcriptomics Data

S. S. Auerbach, NIEHS, Research Triangle Park, NC.

Dose-related changes in gene expression are increasingly being used to characterize the response to chemicals in both in vitro and in vivo systems. Biological potency derived from genomic dose/concentration response approaches can forecast the toxicological potency of a test compound. The quantitative accuracy of this relation has been characterized extensively in in vivo systems and is currently under investigation in in vitro systems. However, characterization of the quantitative aspects of toxicity amounts to an incomplete representation of a chemical’s toxicological properties. The qualitative outcomes, on the other hand, can range from minimal to extreme severity and can impact risk assessment. To address this issue, a data mining exercise was performed. The TC-Gates data set has been employed to identify molecular biomarkers from short-term in vivo studies (1 and 3 days) that forecast a subset of pathological manifestations that showed a dose-related trend in pathological severity from kidney and liver in subchronic toxicity studies (i.e., 28 day pathological outcomes). This exercise Identified several biomarkers previously shown to be markers of kidney (e.g., Hvac2) and liver damage (e.g., Cyp2c3). This approach also allowed the identification of some novel markers that are specific to certain subtypes of pathology. Dose-response modeling of the subset of the biomarker genes from short-duration time points showed they exhibited similar potency to the pathological findings at later time points. These results suggest their potential to serve as surrogate endpoints for estimating a toxicological potency from short-term studies.

1214 Statistical Modeling of the Interindividual Variability of Human Adverse Responses Based on High-Throughput Transcriptomics Data

S. van de Water, Universiteit Leiden, Leiden, Netherlands.

Drug-induced liver injury (DILI) remains a major concern for the clinic and pharmaceutical companies. Therefore the need to improve its prediction at an early phase during drug development. One of the early key events of DILI is the activation of adaptive stress responses, a cellular mechanism to overcome stress. Given the diversity of DILI outcomes, it is key to map the inter-individual variability in activation of these stress responses. Accurately capturing this variance, could aid in the improvement of drug toxicity screening strategies. Therefore, using high throughput targeted RNAseq (TempoSeq), we profiled the transcriptome of a panel of 50 cryo-preserved primary human hepatocytes derived from different individuals exposed for 8 or 24 h to a broad concentration range of tunicamycin, diethyl maleate, cisplatin, and TNFa. These tool compounds were selected to respectively trigger unfolded protein response (UPR), oxidative stress response, DNA damage response, and NF-kB signaling. The variance in the concentration-dependent stress response activation among individuals could be captured, where the average of point-of-departures (PoD) for UPR-related genes resulted in a maximum difference of 866-fold between different hepatocytes. For each stress response, hepatocytes were classified based on a sensitivity score using maximum fold change and PoD for pathway-related genes. The most sensitive and insensitive hepatocytes for UPR or oxidative stress response were further characterized using various hepatotoxicants. Using a population mixed-effect framework, the distribution of the PoDs and maximum fold change were modelled allowing to simulate smaller or larger primary human hepatocytes (PHH) panel sizes. Here, small panel sizes systematically underestimated the variance by 2-fold. This finding indicated that either PHH panel size should be increased or safety factors could be used to obtain improved predictions of the variability and DILI susceptibilities at an early phase of drug development.

1215 Metabolomics-Based Read-Across Approach to Detect Biological Similarity

S. Sperber, BASF SE, Ludwigshafen am Rhein, Germany. Sponsor: M. Leist.

The database MetaMap®Tox is a large collection (>1000 compounds) of plasma metabolome data derived by repeat dose toxicity studies in rats and by different in vitro systems (i.e., liver and kidney cells). Using MetaMap®Tox, the potential toxicity of new compounds, showing biological similarity, was
assessed already during early development. Several examples have shown the actual applications of this approach. For example, metabolomics was applied for read-across using a group of well-studied phenoxy-acetic and -propionic acid chemicals. Here, the best amongst two source compounds was identified using the metabolome data. The metabolomics similarities enabling the read-across were also identified in an in vitro metabolomics approach using the cell lysate of HepG2 cells. Further examples with amino alcohols and plasticizers have shown how metabolomics can support the identification of biological similarity. In this presentation, past and ongoing work will be presented, which illustrate how metabolomics can supply the basis for a substantial read-across hypothesis. The strategy and the used tools to define similarity of chemicals and possibilities to use the data are shown. Principal Component analysis applied to metabolomics data sets combined with structural as well as toxico-dynamic similarity) will be exemplified. In order to increase transparency and paving the path towards regulatory acceptance, numerous activities for quality assurance and validation have been initiated to refine the approach. This includes, e.g., application to reach omics understanding to reporting standards as well as ring trials addressing robustness and reproducibility. A comprehensive discussion including details on this activity will be finally held.

**1216 Impaired Formation of Neural Rosettes from Stem Cells as Teratological Correlate of Toxicant-Induced Transcriptome Disturbances**

N. Dreser. Universität Konstanz, Konstanz, Germany. Sponsor: M. Leist

Many newer generation in vitro teratology tests use human cells and measure mechanistic endpoints including transcriptome changes. However, the toxicological implications of mechanistic parameters are hard to judge without functional or biological endpoints. To address this issue, we developed a new generation of the human stem cell-based test STOP-tox(UKN) Assay. For this purpose, the capacity of the cells to self-organize to neural rosettes was assessed as functional endpoint. Pluripotent stem cells were allowed to differentiate to neuroepithelial cells for six days in the presence or absence of toxicants. Then, both transcriptome changes were measured (standard STOP-tox(UKN)), and cells were allowed to form rosettes. After optimization of staining methods, an imaging algorithm for rosette quantification was implemented and used for an automated rosette formation assay (RoFA). Neural tube toxicants (e.g., valproic acid), which are known to disturb human development at stages when rosette-forming cells are present, were used as positive controls. Established toxicants led to distinctly different tissue organisation and differentiation stages. RoFA outcome and transcript changes, obtained by Affymetrix chip-based DNA microarray analysis, largely correlated concerning (i) concentration-dependence, (ii) time-dependence, and (iii) the set of positive hits identified amongst 24 potential toxicants. The correlation was confirmed by different prediction models based on linear correlations and on random forest. A high throughput version of this teratology test may be used for a simplified and less costly screening of developmental toxicants.

**1217 Application of Cell Painting, an Imaging-Based High-Throughput Phenotypic Profiling Assay for Bioactivity Screening of Environmental Chemicals**

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

Cell Painting is an imaging-based high throughput phenotypic profiling (HTPP) assay that uses fluorescent probes to label a variety of organelles (e.g., nucleus, golgi, mitochondria) and measures a large number of features at the cellular level (e.g., intensity, localization of signal) to detect chemical-induced changes in cell morphology. This assay can be deployed in high throughput (HT) screening format across multiple human-derived in vitro models. The assay can generate high content data (n=1300 measured features/cell) that can be leveraged to identify potency thresholds for perturbation of cellular biology and inform putative mechanism-of-action prediction for next generation risk assessment. Here, we describe HTPP screening assay design and workflows for phenotypic feature extraction. Open-source approaches for concentration-response modeling of high-dimensional data and the evaluation of assay reproducibility using phenotypic reference chemicals will also be debated. Finally, results from concentration-response screening of >1,200 chemicals from the ToxCast chemical library in U-2 OS cells treated for 24 h will be presented. Overall, 41% of the chemicals produced a change in cell phenotype and were classified as hits in the HTPP assay. Where possible, based on the availability of HT toxicokinetic modeling data, micromolar potency values from acute in vitro chemicals in the HTPP assay were converted to administered equivalent doses (AEDs) using in vitro to in vivo extrapolation and reverse dosimetry. For many chemicals, AEDs based on HTPP bioactivity potentials were higher than predicted human exposures and lower or comparable to in vivo effect values from mammalian toxicity studies. Using profile comparison methods, we observed that profiles for retinoic acid receptor agonists (e.g., arottenoid acid, bexarotene) and glucocorticoid receptor agonists (e.g., betamethasone, dexamethasone) were similar to their respective model compounds (retinoic acid, dexamethasone). In addition, profile similarities were observed for several different classes of pesticides (e.g., organochlorines, neonicotinoids). Participants in this session will gain a broader understanding of imaging-based HT profiling methods and potential applications relating to chemical grouping and prioritization in the context of chemical safety assessment. This abstract does not reflect US EPA policy.

**1218 New Approaches for the Identification and Evaluation of Chemical Respiratory Sensitizers**

S. Krieger. Dow, Midland, MI.

Sensitization of the respiratory tract has significant potential health implications; however, the prediction of chemical respiratory sensitizers presents a challenge due to the lack of validated test guidelines and formally recognized assays for this endpoint. This Workshop will bring together representatives from industry, government, and the public sector to (1) discuss the current state-of-the-science for the identification and characterization of chemicals with the potential to cause respiratory sensitization; (2) outline the regulatory and practical needs for hazard identification, risk assessment, and risk management; and (3) describe progress on the development of standard methods and frameworks. The first speaker will provide an overview of respiratory sensitization, what is known of the adverse outcome pathway, where gaps in mechanistic understanding remain, and how those gaps impact the evaluation of chemical respiratory sensitizers. The second speaker will outline recent developments in regulatory approaches for assessment and evaluation of potential respiratory sensitizers. The third speaker will present on recent progress in the development of in vitro methods to identify and discriminate respiratory sensitizers and the needs for further improvements to eventually gain regulatory acceptance. The fourth and fifth speakers will describe the methodology developed to evaluate the potential to cause adverse outcomes using a categorized database of positive and negative respiratory sensitizers that could be used to evaluate in silico and in vitro approaches, including use of human clinical and occupational data in a weight-of-evidence approach. Although recent Workshops have been held to present the state-of-the-science for assessing respiratory toxicity, this Workshop will specifically focus on the adverse outcome of respiratory sensitization and will include a discussion of new approaches for regulatory assessment and evaluation. Attendees will leave with an understanding of the state-of-the-science and currently available tools as well the usefulness and limitations of the presented approaches and where challenges remain. The Workshop will conclude with a roundtable discussion and Q&A between the presenters and attendees.

**1219 Chemical Respiratory Sensitizers: Current Understanding and Remaining Gaps**

S. Krieger. Dow, Midland, MI.

Certain low molecular weight (LMW) chemicals have the ability to induce allergic sensitization of the respiratory tract and the potential to cause adverse outcomes; therefore, accurate identification of these substances is an important public health issue. As with dermal sensitization, respiratory allergy occurs in two phases: (1) induction (sensitization) which includes initial inhalation or dermal exposure to the allergen and development of the immune response, and (2) elicitation, the symptomatic phase. Despite this similarity, there are important differences in the mechanisms whereby respiratory and dermal sensitization develop: (1) respiratory allergy typically occurs via an antibody-mediated response whereas dermal sensitization is most often cell-mediated, and (2) the functional subpopulation of T helper (Th) cells primarily involved in development of respiratory sensitization is the Th2 population of T lymphocytes, whereas for dermal sensitization Th1 cells are preferentially induced. The adverse outcome pathway (AOP) for dermal sensitizers has been clearly defined, and well-established assays exist to identify these substances. While the key events in the AOP for the induction phase of respiratory sensitization have been identified, the molecular and cellular mechanisms that result in sensitization of the respiratory tract are poorly understood, and currently, there are no validated or regulatory accepted assays for the identification of respiratory sensitizers. Due to the inability to predict a priori the respiratory sensitization potential of substances, regulatory evaluation of respiratory sensitizers is often based on weight of evidence (WoE) approaches, with consideration of human reports as well as data from in vitro and in vivo studies, structural alerts, (Q)SAR, and read-across. Improvement in the understanding of the molecular, cellular, and physiological aspects of respiratory allergy will aid in development of testing approaches to distinguish
dermal and respiratory sensitizers and non-sensitizers and allow for accurate identification of LMW chemicals with respiratory sensitization potential. In order to develop integrated assessment approaches to identify agents that elicit this condition, a collaboration of current research efforts and regulatory evaluation approaches will be needed.

**W 1220 Evaluations for Chemical Respiratory Sensitizers under Section 5 of the Amended Toxic Substances Control Act (TSCA)**


In June 2016, the Frank R. Launtenberg Chemical Safety for the 21st Century Act was signed into law, thereby amending the Toxic Substances Control Act (TSCA), the nation’s primary chemicals management law. The amendments placed new requirements on the US EPA (EPA), including for example, utilizing non-vertebrate testing methods, when scientifically justified, to support determinations as to whether new chemical substances present unreasonable risk under Section 5 of TSCA. Since no validated test guideline is available for assessing chemical respiratory sensitizers, EPA may utilize non-vertebrate test data or existing vertebrate test data to assess this endpoint, which may occur following dermal or inhalation exposures, using a weight of evidence approach, which includes evaluating the physical/chemical properties, reviewing available skin sensitization data, identifying structural alerts and/or anticipated metabolites, assessing the potential for protein cross-linking, etc. The goal of these evaluations is to estimate the bioavailability and reactivity of the new chemical substance and potential metabolites and to make a determination on whether or not the new chemical substance may present a hazard concern for respiratory sensitization. The purpose of this presentation is to: (1) inform participants on the types of information sources that EPA utilizes for performing these evaluations, (2) communicate the types of determinations that may be made, if EPA concludes that a new chemical substance may be a respiratory sensitizer, and (3) explain the process for discussing the acceptability of non-vertebrate test methods with EPA, prior to initiating testing.

**W 1221 Current Status of In Vitro Models to Identify Respiratory Sensitizers**

A. Gutleb. Luxembourg Institute of Science and Technology, Belvaux, Luxembourg.

Inhalation of chemicals can lead to a wide range of effects from irritation in the airways, induction of respiratory sensitization, to systemic effects. *In vitro* cell culture models representing different parts of the pulmonary system have been developed in recent years and, individually, cover the full anatomy of the airway from the nasal mucosa to the alveolar region. Such models range in complexity from single cell-lines to complex 3D models consisting of several different cell lines or primary cells. Some models for the nasal, tracheal, bronchial tract and the bronchioli are commercially available. However, currently there are no models relevant to sensitization are less well-developed than for other effects, and only few models show promising results. The most promising models have different approaches to reflect biological complexity. One approach uses an artificial intelligence algorithm applied to the expression pattern of a set of 28 genes upon exposure of a single cell type, this model has shown promise with correct identification of several known sensitizers, although in a kind of black-box approach. A second type of model is based on more complex *in vitro* models cultured in a 3D orientation at the air-liquid interface (ALI). The model consists of several cell types, including alveolar cells, endothelial cells and immune cells, mimicking the alveolar barrier as the site of high relevance for the induction of respiratory sensitization. Endpoints analysed in this model consist of surface cell types, including alveolar cells, endothelial cells and immune cells, mimicking the alveolar barrier as the site of high relevance for the induction of respiratory sensitization. Endpoints analysed in this model consists of surface markers (CD54, CD86, TSLP, etc.), gene expression (IL1RL1, etc.) and excretion of cytokines (CCL20, GM-CSF, etc.). Again the identification of a respiratory sensitizer is based on the pattern of changes in the endpoints. The status of development, validation, and needs for further improvements of such *in vitro* models with the aim to identify respiratory sensitizers, and if possible to predict their potency, will be discussed.

**W 1222 Development of a Reference List of Chemical Respiratory Sensitizers to Facilitate Evaluation of Integrated Approaches to Testing and Assessment**

K. Sullivan. Physicians Committee for Responsible Medicine, Washington, DC.

Despite the progress made with and high regulatory need for developing hazard identification approaches for assessing the potential for chemicals to cause respiratory sensitization, no harmonized approaches have progressed towards regulatory acceptance. In order to assess potential *in silico* and *in vitro* approaches for the detection of chemical respiratory sensitization, a reference list of ~120 potential chemical respiratory sensitizers was generated based on structural alerts of likely sensitizers. We followed a weight of evidence (WoE)-based approach to build the reference list with publicly-available toxicity data, (Q)SAR predictions, and other information such as physical/chemical properties, metabolic transformation (if known), and presumed binding mechanisms. To allow for a WoE analysis, we have collected all data and are qualifying the data to reflect this methodological quality. We are also making use of the Abstract Sifter literature review tool to identify additional potential respiratory sensitizers. The resulting reference list, which will be finalized in consultation with relevant regulatory agencies and made available via a public database, is an important step towards the assessment of potential test methods and the creation of internationally harmonized integrated approaches for the identification of chemical respiratory allergens.

**W 1223 Utilization of Human Evidence for Testing and Assessment of Chemical Sensitizers**

R. Rajagopal. Unilever Safety and Environmental Assurance Centre, Sharnbrook, United Kingdom. Sponsor: S. Krieger

Tapping into clinical and epidemiological data can be a relevant and reliable approach into chemical hazard and risk assessment for both Chemical Respiratory Allergy (CRA) and Allergic Contact Dermatitis (ACD). An approach was undertaken for utilization and curation of human data towards validating the reference list of putative respiratory sensitizers. This approach took into consideration information on exposure history, the vapor pressure, diagnostic tests used, uncertainties associated with these analyses, reporting of results, and other potentially confounding factors, through well-defined criteria and a specific scoring matrix, to make a decision on whether a chemical has caused respiratory sensitisation or not. This approach yields a YES, NO or EQUIVOCAL outcome, with respect to sensitizing potential, depending on whether the set criteria were fulfilled, not fulfilled or only partially fulfilled, respectively, and can serve as a guidance for determining chemical hazard for novel chemical risk assessments. Similarly, recent experience with skin sensitization new approach methodologies has demonstrated that the predictivity of next generation risk assessment (NGRA) approaches can be benchmarked through expert review and statistical modelling of human dermatology datasets. This concept utilized historical clinical experience to establish exposures for skin sensitizing chemicals which are considered as either high or low risk for induction of skin sensitisation. Comparing novel chemical exposure with these established benchmarks can be used to provide part of its risk of inducing skin sensitization. This work draws parallels from these novel experiences to propose how human evidence can be used to inform and benchmark integrated approaches to testing and assessment.

**W 1224 Tackling the Potential Human Health Impacts of Microplastics and Nanoplastics: Challenges for Toxicologists in the Assessment of Real-World Complex Mixtures**

N. Walker. NIEHS/NTP, Research Triangle Park, NC.

Micro- and nanoparticles are particles that are formed from the breakdown of bulk plastic waste and, in some cases, intentionally synthesized for commercial use. The degradation process of bulk plastics produces visible particles to sub-visible particles and ultimately nanoparticles with mixed compositions, shapes, and sizes. There are no universal consensus standards for the definition of micro- and nanoparticles, but microplastics are generally considered to be in the range of 5 mm to 1 micron and nanoparticles are considered to be less than a micron in size. With the annual global accumulation of millions of tons of plastics in oceans, rivers, soils, plants, and sediments, the formation of these micro- and nanoparticles will only increase in the future. Less than 15% of plastics produced globally are recycled according to some estimates, since they are inexpensive to manufacture but expensive to segregate and recycle plastic waste products. Even though the concern about plastic waste has existed for many years, the recent awareness of the increased presence
of micro- and nanoplastics in the air we breathe and their detection in food, seafood, and water, resulting in human exposure, is causing public concern. While microplastics can be observed with light microscopy and quantified, measurement methods for sub-micron-size nanoplastics mixtures are also not available to conduct a thorough analysis, to aid in human exposure assessment, due to the myriad challenges associated with their collection, separation, isolation, and characterization of real-world mixtures from environmental and complex organic matrices. Lessons learned from pitfalls in nanomaterial assessment and assessment of hazard of complex mixtures can be utilized to address these challenges with complex nanoparticles mixtures.

While this global problem has been brewing for decades, there is no current definitive evidence of human toxicity. The questions one would pose are (1) whether this is a real problem, and (2) if so, how to address this through appropriate studies with real-world samples, rather than "model compounds" that cannot capture the potential toxicity resulting from the complex mixtures. What makes it challenging is that unlike engineered nanomaterials, these incidental nanoplastics are not uniform and contain sizes ranging from nano to micron, with various chemical compositions along with chemicals adsorbed to them. This Workshop will bring multiple sector (government, academia, industry, non-profit) experts to present on specific issues to keep in mind when exploring toxicological research related to the complex real-world samples microplastics and nanoplastics mixtures. The topics that will be covered include (1) various compositions and risks of contamination of microplastics collected from different parts of the oceans and water bodies; (2) challenges one would face in isolation and characterizing various aspects of real-world samples, collaborating efforts from government agencies; (3) case study examples, lessons learned, and toxicological assessment approaches from tire-wear debris mixtures; (4) challenges in predicting exposure; and (5) potential human health impacts of both micro- and nanoplastics.

**W 1225 Overview**

**N. Walker, NIEHS/NTP, Research Triangle Park, NC.**

Even though the concern about plastic waste existing for many years, the recent awareness of the increased presence of micro and nanoplastics in the air we breathe, their detection in food, seafood, and water, resulting in human exposure, is causing public concern. While this global problem has been brewing for decades, there is no current definitive evidence of human toxicity. The questions one would pose are (1) whether this is a real problem, and (2) if so, how to address this through appropriate studies with real-world samples, rather than 'model compounds' that cannot capture the potential toxicity resulting from the complex mixtures. What makes it challenging is that unlike engineered nanomaterials, these incidental nanoplastics are not uniform, contain sizes ranging from nano to micron, with various chemical compositions along with chemicals adsorbed to them. This Workshop will bring multiple sector (government, academia, industry, non-profit) experts to present on specific issues to keep in mind when exploring toxicological research related to the complex real-world samples microplastics and nanoplastics mixtures. The topics that will be covered include: (1) various compositions and risks of contamination of microplastics collected from different parts of the Oceans and water bodies; (2) challenges one would face in isolation and characterizing various aspects of real-world samples, collaborating efforts from government agencies; (3) case study examples, lessons learned, and toxicological assessment approaches from tire-wear debris mixtures; (4) challenges in predicting exposure; and (5) potential human health impacts of both micro- and nanoplastics.

**W 1226 What Are the Risks of Global Contamination by Microplastics?**

**K. Lavender Law. Sea Education Association, Woods Hole, MA. Sponsor: N. Walker**

Environmental pollution by microplastics has quickly become a serious concern to the public, the media, policymakers and scientists because of their widespread occurrence and potential impacts to wildlife and human health. As early as the 1970s, small plastic fragments and industrial resin pellets were found drifting in the open ocean. By the 2010s, microplastics — typically defined as plastic particles smaller than 5 mm in size — had been detected throughout the marine, freshwater and terrestrial environment, and also in many items commonly consumed by humans. Microplastics are abundant and widespread in the natural and built environment, largely originating from the fragmentation of larger plastics (in the case of synthetic fibers) of larger from. The nascent study of microplastics, and even smaller nanoplastics, is challenged by lack of standard definitions and measurement protocols arising from the diversity and complexity of this category of contaminants: a microplastic is not a microplastic. Further, the physical and chemical characteristics of these particles evolve in response to environment.
found in human stool indicating exposure. MPs have been found in drinking water, beverages (e.g., beer, milk, tea, and soft drinks), table salt, and seafood. However, many of the reports do not include the smallest MPs in their analysis, which is important since the smallest MPs may be taken up in the intestinal tract. 5) Lack of reference materials for testing/developing analysis methods, and evaluating health effects. Most published work has been with polystyrene spheres. A few plastics are of toxicological concern in drinking water and food products: polyethylene, polyethylene terephthalate, and polypropylene. No MPs are commercially available for any of these three plastics, and only few larger MPs are available. 6) Limited capabilities of measuring NPs and MPs in biological samples. Analysis methods for small particulate materials are specialized and not widely available. These include pyrolysis GC-MS, Raman spectroscopy, FTIR. NPs and small MPs present methodological challenges for extraction, characterization, and identification. While much of the research to date on NPs and MPs has focused on marine life, we will discuss the components that need to be assembled to move forward with assessment of exposure.

1230 Strategic Directions and Research Needs for Assessing Human Health Impacts of Micro- and Nanoplastics


Microplastics are environmentally-persistent pollutants characterized by their general composition and size range. However, microplastics are an extremely diverse set of materials. Sources and chemistry range widely, including manufactured microbeads, particles from tires, polyester fibers from clothing and carpets, and weathered particles from disposable consumer products. Traditionally, microplastics have been defined as smaller than 5 millimeters in diameter, but particles in the range of a few microns or smaller are most likely to be bioavailable to humans. There is extensive research on microplastics in the ocean, and potential human exposure through seafood, but there are major gaps in the information needed to conduct a multi-pathway exposure assessment. For example, little research has been done on the indoor environment, where microplastic particles and fibers abound in air and dust. The body of science on the human health effects of particulate matter and nanomaterials offer lessons that can be applied to microplastics. Some relevant considerations include the composition of the plastic (including additives and adsorbed contaminants), shape, density, and surface chemistry. The array of variables, and uncertainty as to which may be most relevant to human health has led to special research challenges. Few toxicology studies have been conducted, and the design of such studies poses additional challenges. For example, in vitro assays may not be feasible for these materials. The fact that microplastic particles are now widespread in our environment makes it urgent to assess human exposures and health effects.

1231 Thresholds of Toxicological Concern: Reassessing the Basis and Expanding the Horizon

H. M. Hollnagel. Dow Europe GmbH, Horgen, Switzerland.

This session will provide an update on the state of the art of thresholds of toxicological concern (TTC) as a springboard for where this concept will go in the future. The TTC concept is an approach implementing de minimis thresholds to prioritize chemicals for hazard characterization and risk management. The origins of the TTC stem from the US FDA’s Threshold of Regulation (FDA, 1995), which was developed as a tool to facilitate the safety evaluation of food packaging materials, components of which might have the potential to migrate into food at very low levels. It has since been expanded from a single value (the FDA Threshold of Regulation) to encompass a range of exposure limits based on potency bins for chemicals. The approach has been endorsed by various global regulatory agencies for the assessment of flavors, food contact materials, drug impurities, cosmetic ingredients, and beyond. Large datasets of cancer and non-cancer potency information underpin the TTC concept. While the basic principles of hazard characterization based on animal studies have hardly changed, testing guidelines have been updated and new studies have become available. In addition, there are greater opportunities to apply mathematical approaches to derive structure-toxicity relationships, as well as new approach methodologies, which allow for incorporation of mechanistic or toxicokinetic information. The overall TTC concept has, if anything, become even more relevant due to the ever-increasing sensitivity of analytical methods, the need to prioritize large numbers of substances, and the desire to avoid animal testing. Recent expansion and reassessment of the two key toxicity datasets underpinning TTC—the cancer potency and non-cancer datasets—to include additional chemicals and studies will be described along with the evaluation of the points of departures.

For cancer potency, the focus was to substantiate the TTC exposure limits for compounds considered to pose a possible DNA-reactivity hazard versus those that do not by investigating the influence of DNA-reactivity on potency as well as refining risk estimates comparing benchmark dose levels (BMDLs) with carcinogenic potencies expressed as TD50s. Projects to expand the chemical applicability domain of non-cancer datasets (e.g., by the inclusion of antimicrobials) and to derive TTC thresholds based on blood concentrations rather than external oral dose (internal TTC [iTTC]) to enable applicability to systemic toxicity via additional routes of exposure will also be described. Beyond the toxicity databases, novel concepts on how to group chemicals into structural categories, linked to potency groups for the de minimis thresholds, will be presented. Tradition for the threshold derivation by application of the Cramer et al. decision tree from 1978, which has been judged by regulatory agencies to deliver fit-for-purpose classification for TTC threshold derivation. However, the outcomes from two complementary projects will be highlighted to show how the Cramer decision tree (CDT) has been updated by expert knowledge versus applying a cheminformatic approach to devise structural categories. The range of diverse TTC projects demonstrate the multiple facets of hazard characterization with novel and traditional approaches. The session will provide an overview of the current status of TTC application, including limitations of technologies, and highlight good practice for regulatory-relevant databases in interpreting the results from in vivo studies, selection of appropriate results, transparency, and assessment of DNA reactivity by expert judgment, SAR, or read-across, as well as grouping structures and deriving TTC, including a variety of sources of variability, bias, and uncertainty. Finally, the session will provide a vision of how TTC can be used within risk assessment and regulatory frameworks. Specifically, the uncertainties behind TTC will be characterized and methods to overcome high uncertainty, and thus improve confidence, will be presented. These, in combination with the new developments in TTC presented in the session, will demonstrate the increasing transparency in the concept, which can form a valuable part of Next Generation Risk Assessment.

1232 The New Cancer Potency (Updated CPDB) Database for TTC

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Substances posing a real or potential DNA-reactivity hazard are assigned to the TTC category with the most stringent exposure limit of 0.0025 ug/kg bw/ day (0.15 ug/day for a 60 kg adult). This exposure limit, first published by Kros et al., was based on the distribution of cancer potencies for over 730 carcinogens and has been widely accepted in regulatory opinions on TTC. Boobis et al (2017) recommended a review to update the existing database of carcinogens that was evaluated when this exposure limit was first established. Here, the original compilation was augmented by the addition of new data for ~60 compounds from the National Toxicology Program (NTP), European Food Safety Agency (EFSA) Draft Assessment Reports (DARS) and other publicly available sources. A thorough curation and quality control of existing and new data was made to a common standard. The new database is intended to support the re-evaluation or development of TTCs for carcinogens. Compounds were assessed based on potential DNA-reactive or non-DNA-reactive carcinogenicity using either existing in vitro and in vivo genotoxicity (mutagenicity or clastogenicity) data (where available) or in silico models using structural alerts and QSARs for DNA reactivity. Points of Departure were calculated as both TDS0 and/or MBDLs, depending on the suitability of the data for BMD modelling. The updated CPDB database and all supporting information will be made freely available as an electronic spreadsheet. Using data extracted from the database, updated TTC values are being explored to examine the applicability of the Cramer decision tree thresholds. Further, the database has been made available for another complementary project to investigate if new TTC values for non-DNA-reactive carcinogenicity might be warranted. The funding of CEFC Project LRI-B18 is gratefully acknowledged.

1233 US FDA’s Updates to the Cramer et al. (1978) Decision Tree: The Expanded Decision Tree

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The Cramer et al. (1978) Decision Tree (CDT), a screening and prioritization tool, sorts chemicals into three classes of relative toxicity. Given the scientific advancements and collective understanding accumulated since 1978 and the exponential increase in the number and types of chemicals to which humans are known to be exposed, the CDT has been long overdue for an update. The US FDA updated and
expanding the CDT to reflect the current state of the science and to make it applicable to a much broader scope of substances present in food, food contact materials, cosmetics, dietary supplements, and, elsewhere. More than 18,000 scientific studies were reviewed to determine the effects of species, strain, sex, and target organ on toxicity and metabolic fate. Based on these studies, over 1,900 substances were then organized according to their structure, metabolic potential, and potential toxicity. Analytical tools that can be used to convert the chemical specific external dose to an internal TTC (iTTC) requires a significant amount of data and computational tools that can be applied more widely. One of the next steps in the continued evolution of the iTTC approach as compared to a substance-specific risk assessment. In a first phase of the project the focus was on qualitative description and ranking of the identified sources of uncertainty (UC). Later, these UC will be assessed quantitatively. UC associated with the development of the TTC values include, for example, AM categories derived in this study were stratified across potency classes to classify AMs beyond the capability of the conventional Cramer decision tree. This quantitative grouping approach liberates users from the current paradigm confined by pre-determined classifications. This talk presents an overview of the development of a new framework for potential assessment of chronic toxicity, empowering users with modern reliable database and chemoinformatic methods.

**1234 A New Paradigm in Threshold of Toxicological Concern Transforming Cramer Class Approaches to Chemotype Grouping Based on Chemoinformatics Analysis of a Highly Curated Database**

C. Yang, MN-AM, Nürnberg, Germany.

A new large database to derive highly curated data sets for the Threshold of Toxicological Concern (TTC) approach has been compiled by consolidating existing TTC datasets. The database has been enriched with antimicrobial (AM) chemicals as well as food and cosmetics ingredients, resulting in 1357 chemicals with 276, 54, and 1027 substances in Cramer Classes I, II, and III, respectively. The chemical space enrichment was achieved by establishing a regulatory antimicrobial inventory (681). Strict toxicity data fusion rules were applied in the curation process. Pre-determined classification systems such as Cramer classes have limitations in addressing a broad spectrum of chemical types. Although a large fraction of antimicrobials is Class III, whose threshold was determined to be approximately 0.12 mg/kg-bw/day, their structures and no observed adverse effect level (NOAEL) values vary widely. Based on the highly curated dataset, a new chemoinformatics method for grouping chemicals and associating chemotype categories with potency was developed. As an example, AM categories derived in this study were stratified across potency classes to classify AMs beyond the capability of the conventional Cramer decision tree. This quantitative grouping approach liberates users from the current paradigm confined by pre-determined classifications. This talk presents an overview of the development of a new framework for potential assessment of chronic toxicity, empowering users with modern reliable database and chemoinformatic methods.

**1235 Working toward the Development of an Internal Threshold of Toxicological Concern (ITTC)**


UC were identified as major barrier for UC quantification. In addition to the datasets to understand variability, UC in knowledge on variability, and other factors, choice of the point of departure (NO(A)EL/ TD50), overall database quality, and the choice of the 5th percentile of the NOAEL distributions for threshold derivation of non-DNA-reactive compounds. UC related to the application of TTCs in risk assessment are: chemical space covered by the structures in the database, identification of excluded substance groups, use of in silico predictions of mutagenicity, the applicability of one TTC value to cover different toxicological endpoints (repeated dose toxicity, Developmental and Reproductive Toxicity (DART) etc.), and the influence of Cramer Class misclassification. The estimated level of UC was similar for some factors, irrespective of whether the risk assessment is based on TTC or substance-specific data. The ILSI Europe Expert Group modified some of the current methodology to estimate and describe separately the magnitude of the potential impact a UC would have on an assessment and the likelihood that such UC might be relevant, to achieve more transparency. Lack of availability of suitably large data sets to understand knowledge on variability, and other UC were identified as major barrier for UC quantification. In addition to the direct determination and ranking of UC associated with application of the TTC approach, the project provides a useful example of method selection and challenges in UC characterization, more generally. Such case studies are very timely, due to the demand for greater transparency in risk assessment and better communication of the attendant uncertainties to risk managers.

**1236 Characterizing Uncertainty in TTC**


The probabilistic approach using the cancer and non-cancer Thresholds of Toxicological Concern (TTC) to assess for concerns from exposure to data poor compounds is often perceived as accepting a higher risk than traditional assessments. An ILSI Europe Expert Group was convened to characterize and quantify the uncertainties associated with the application of the TTC approach as compared to a substance-specific risk assessment. In a first phase of the project the focus was on qualitative description and ranking of the identified sources of uncertainty (UC). Later, these UC will be assessed quantitatively. UC associated with the development of the TTC values include, for example, AM categories derived in this study were stratified across potency classes to classify AMs beyond the capability of the conventional Cramer decision tree. This quantitative grouping approach liberates users from the current paradigm confined by pre-determined classifications. This talk presents an overview of the development of a new framework for potential assessment of chronic toxicity, empowering users with modern reliable database and chemoinformatic methods.

**1237 The Community Exposure: Effects of Environmental Contamination on Health Disparities and Marginalized Populations through the Lens of a Toxicologist**

J. Zelikoff. New York University, New York, NY.

Environmental justice communities, “poor and minority communities that bear a disproportionate burden of environmental health risk,” not only face societal and psychosocial stressors, but are also unjustly exposed to disproportional levels of environmental contaminants. Environmental contamination and socioeconomic and racial disparities in disease burden have been a long-standing public health problem with historical roots. The current COVID-19 pandemic has brought this issue to the forefront and increased public awareness of these disparities. Adding to the pressure posed by these social inequities are environmental factors such as air and water quality that plague these marginalized communities and contribute extensively to their vulnerability and disproportionate disease burden. This Workshop will discuss how environmental contamination and exposure contributes to the health dispar-
ities associated with marginalized populations, including Native Americans and urban communities, using specific case studies. Existing state and federal policies and the regulations currently in place and needed to protect such vulnerable populations also will be presented, along with possible solutions and suggestions of how to move the field of environmental health equity forward in our post-COVID world. The presentations will be followed by a question and answer panel discussion with all the participants.

1238 The Nature of the Problem

C. Jackson. NIEHS, Research Triangle Park, NC. Sponsor: J. Zelikoff

Human health may be affected by a variety of factors, including diet and toxic substances. According to Dr. Jackson, the definition of environment should also include the social circumstances in which people live, work, play, and worship. She believes that social factors, including poor living and working conditions, as well as experiences of racial discrimination, can contribute to increased risk of obesity, hypertension, type 2 diabetes, and cardiovascular disease. Dr. Jackson will open the program with an overview of historical and contemporary environmental injustices that are relevant to the toxicology community. Using sociocultural and biopsychosocial models as frameworks, Dr. Jackson will describe how upstream factors in the physical (e.g., air pollution, personal care products, chemical contaminants) and social (e.g., racism) environments are believed to drive disparities in under-resourced, marginalized populations. In addition to identifying the biological mechanisms by which factors in the physical and social environments affect health and contribute to health inequities, she will discuss the translation of epidemiologic findings into novel environmental interventions and practices that address structural, macro-level, as well as individual-level barriers to achieving and maintaining optimal personal and environmental health.

1239 Native American Populations Face a Legacy of Environmental Contamination, Marginalization, and Health Disparities: Story of the Ramapough Lunamee Turtle Clan Nation

V. Mann. Ramapough Lunamee Nation Turtle Clan, Ringwood, NJ. Sponsor: J. Zelikoff

For Native Americans, a disproportionately greater risk of adverse health outcomes, such as cardiovascular and kidney disease, asthma, and diabetes can be largely attributed to multi-generational poverty and political marginalization. Given that over 400,000 Native Americans live within 3 miles of a contaminated waste dump or EPA-listed Superfund site, they also experience cumulative environmental exposures including poor food, water, and air quality, which likely exacerbate pre-existing health disparities associated with low socioeconomic status. Many epidemiological studies have examined health implications of Native American tribal members’ exposure to Superfund sites and associated contaminants located on or near their land. For example, evidence of elevated prevalence or incidence of type II diabetes, breast cancer, and/or neurological deficits have been observed among the Mohawk at Akwesasne, the Gila River Indian Community and the Alaskan Yupik tribe, all of whom have been exposed to persistent organic pollutants released by nearby industrial facilities, extensive application of pesticides on agricultural land, or former U.S. military bases. This presentation will provide a case history of the Ramapough Lunamee Turtle Clan Tribal nation (located primarily in the Ramapo Mountains of New Jersey and New York state). As a result of industrial dumping between the 1960s and 1970s, the Turtle Clan nation has lived between 0.5 to 2 miles away from a heavily contaminated industrial dump site in Ringwood, NJ; where they were exposed to tons of toxic and carcinogenic paint sludge constituents, solvents, and semi-volatile organics. Throughout the decade of chemical dumping in nearby mines, open pits, or burial in corroding containers, self-reported acute health effects resulting from government actions. While the law was designed to underscore certain provisions of existing law that can help ensure that all communities and persons across this Nation live in a safe and healthful environment – that is not always the outcome. Each Federal agency shall ensure that the public, including minority and low-income communities, has adequate access to public information relating to human health or environmental planning, regulations, and enforcement when required under various congressional statutes and laws. These groups are provided the opportunity to share in the benefits of, and be or not be excluded from, and not affected in a disproportionately high and adverse manner, by federally instituted programs and activities. All federal government agencies whose responsibility are in part to ensure equality have created an internal checks and balances via an office of environmental justice or other program(s) which are based on this principle. A number of agencies have instituted policies, programs and other activities directed to the education and protection of protected classes. For instance, the USDA has a dedicated EJ Strategic Plan which further outlines specific challenges experienced by impacted groups and develops the collaborative efforts to direct nearly $24 billion into investments to create jobs, build homes, feed children, assist farmers, and conserve natural resources in the Nation’s most economically challenged rural areas. This presentation will also discuss what considerations have been given by Agencies regarding the programs and activities that impact marginalized communities and will provide future outlooks on the impacts of such programming as it relates to the impacted parties.

1240 Case Studies from Columbus, Ohio: Place Matters with regard to Health Care Disparities and Disparate Health Outcomes

D. Hoos. Ohio State University, Columbus, OH.

While Columbus, Ohio is considered one of the more prosperous, well-educated and progressive communities in the United States, it has the second worst life expectancy at birth by metro area and one of the highest infant mortality rates in the country. As a result, there are several high risk and vulnerable neighborhoods in Columbus, Ohio and much of our research foci over the past seven years has been directed at understanding the disparate health outcomes that persist in these neighborhoods. In 2013, community leaders in the most impacted neighborhood met with environmental health scientists at the Ohio State University to address concerns related to negative health outcomes being linked to potential exposures to chemical and non-chemical stressors from the built, natural, physical, and social environment in their census tracts. Through the South Side Health Advisory Committee, a community-academic-local state agency partnership emerged that conducted pilot hazard assessments that have matured into a demonstration project. This case study will detail the establishment of a functional, multidisciplinary, community-based research stakeholder team to conduct toxicology research in high-risk communities. As illustrated by this case study, community-led coalitions in collaboration with academia and local public health policy-making officials can effectively address concerns of potential exposure to environmental contaminants in residents from high-risk communities. This case study presentation also incorporates research focused on assessing concordance between health outcomes in high-risk census tracks with COVID-19 related disparate health outcomes. Most importantly, this case study from Columbus, Ohio shows how to estimate the extent to which components of the built, natural, physical and social environment influence the trajectory to COVID-19 disparate health outcomes using our novel Public Health Exposome framework and Big Data to Knowledge (BD2K) analytics.

1241 Environmental Justice Problems of Concern to Disadvantaged Communities: A Regulatory Perspective and Future Directions for Environmental Health Equity

M. King. USDA Agricultural Research Service, Washington, DC.

Executive Order 12898 provides direction to all federal agencies to incorporate Environmental Justice (EJ) concerns into their existing programs. Environmental Justice is a mandate to change or make the difference in the lives of those who could be or have been adversely impacted by environmental effects resulting from government actions. While the law was designed to underscore certain provisions of existing law that can help ensure that all communities and persons across this Nation live in a safe and healthful environment – that is not always the outcome. Each Federal agency shall ensure that the public, including minority and low-income communities, has adequate access to public information relating to human health or environmental planning, regulations, and enforcement when required under various congressional statutes and laws. These groups are provided the opportunity to share in the benefits of, and be or not be excluded from, and not affected in a disproportionately high and adverse manner, by federally instituted programs and activities. All federal government agencies whose responsibility are in part to ensure equality have created an internal checks and balances via an office of environmental justice or other program(s) which are based on this principle. A number of agencies have instituted policies, programs and other activities directed to the education and protection of protected classes. For instance, the USDA has a dedicated EJ Strategic Plan which further outlines specific challenges experienced by impacted groups and develops the collaborative efforts to direct nearly $24 billion into investments to create jobs, build homes, feed children, assist farmers, and conserve natural resources in the Nation’s most economically challenged rural areas. This presentation will also discuss what considerations have been given by Agencies regarding the programs and activities that impact marginalized communities and will provide future outlooks on the impacts of such programming as it relates to the impacted parties.

1242 Toxicology for Chemists: Preparing Chemists to Design Safer Products through Healthier Molecular Design

A. S. Cannon. Beyond Benign, Wilmington, MA.

We live in a time of converging trends where chemists and toxicologists need to work together to understand the toxic effects of chemicals. While significant progress has been made in studying how chemicals impact human...
health and the environment, there is still a lack of proper training among chemists to understand how toxicology can be incorporated into curriculum such that it prepares the next generation of chemists for this transdisciplinary career. There is a movement toward teaching toxicology concepts to chemistry students within chemistry courses and programs but despite this movement, toxicology principles remain a key missing piece to a chemist’s education if designing safer products. Textbook authors and publishers are starting to include chapters on how to use and generate hazard of hazardous chemicals. Some academic institutions have begun efforts to create their own courses on toxicology, or weave concepts into existing chemistry courses. Many institutions have shown interest in this area but do not have the resources or knowledge base to implement toxicology-weighted course or program for future chemists. These departments seek to integrate toxicology into their courses and programs, there is a growing demand for educational materials and models for adoption. Beyond Benign, a nonprofit organization dedicated to Green Chemistry education, has partnered with a team of professional toxicologists and chemistry college and university faculty members to design an open-access curriculum for use within college and professional chemistry education programs. This session will serve as a means for sharing the Toxicology for Chemists curriculum and program and identifying collaborative opportunities for SOT members and chemistry educators to address a growing need for toxicology training in the chemistry curriculum. The goal of the Toxicology for Chemists program is to provide chemists with proper training on foundational principles of toxicology and mechanistic understanding of how chemicals impact human health and the environment. The key audience for this Informational Session is SOT members who are interested in contributing to the education and training needs of chemists, along with chemists involved in designing sustainable chemicals. Dr. Amy Cannon will kick off the session with an overview of the Toxicology for Chemists program. Two members of the program advisory group and curriculum contributors, Dr. Margaret Whitaker and Dr. Bryan Brooks, will present on methods and tools for better educating chemists on toxicity topics. Dr. John Warner, a founder of the field of Green Chemistry and co-author of the defining 12 Principles of Green Chemistry, will close the session by highlighting the opportunities for toxicology to provide chemists with better, smarter design skills and outline specific examples of chemical products designed by using the tools of toxicology.

1243 Opportunities for Human-Induced Pluripotent Stem Cell–Derived Neurons in In Vitro Neurotoxicity Safety Testing

A. Tuukk. Universiteit Utrecht/Purdue University, Utrecht, Netherlands.

Human induced pluripotent stem cell (hiPSC)-derived neuronal cultures provide an excellent opportunity to model the central nervous system in vitro. These cultures are classified as a good alternative to in vivo experiments that have questionable human relevance, potentially due to inter-species differences. Data obtained with hiPSC-derived neuronal cultures might be more predictive of the human response to chemical exposure because using these cells circumvents interspecies translation. Also, these neurons allow for the patient-specific risk assessment and drug testing as they maintain the donor’s unique genetic blueprint. Because they reflect genetics unique to each donor, they allow for modeling of specific diseases. For example, hiPSC-derived neuronal cell lines have been made from donors with neurodegenerative diseases such as Parkinson’s or Huntington’s disease. In light of the aforementioned advancements, the use of hiPSC-derived neurons is rapidly increasing. More and more research groups create their own hiPSC-derived neuronal cell lines. Over the past years, an expanding palette of hiPSC-derived neurons (and astrocytes) has become commercially available, saving researchers the time of performing the differentiation themselves, allowing for rapid assay development and translation of chemical safety testing in vitro. hiPSC-derived neurons are ethically debated and require inter-species translation. These models are therefore familiar with needs of different sectors. This talk will present work being done on complex multicellular 3D systems made from hiPSC-derived neurons and supporting cells and investigating their susceptibility to pesticides. High-throughput testing opportunities will be discussed as well as how academia and industry can help each other in progressing towards more high-throughput systems. The third talk will look at these model systems from a governmental regulatory perspective. What requirements do these cells need to meet before they can be incorporated in a governmental regulatory framework used, for example, in food safety regulations, and how do these regulatory authorities see alternative approaches now? The final talk will describe the criteria that these models need to meet from a pharmaceutical perspective. In this presentation, the speaker will address what pharmaceutical companies want to see before they incorporate hiPSC-derived neuronal models systems in their drug developmental test battery. He will outline how hiPSC-based models fit in neurotoxicity safety assessment and how they compare with the existing animal models. Presentations are followed by a panel discussion on future activities to evolve alternatives to meet regulatory and research needs. At the end of the session, it will be clear where we stand and what steps must be taken before hiPSC-derived neurons are accepted in the regulatory framework and incorporated in the drug developmental pipeline. The ultimate goal is to give perspectives on whether these neurons can replace or reduce in vivo tests.

1244 Human iPSC-Derived Neuronal Models for Neurotoxicity Testing and in Vitro Seizure Liability Assessment

A. Tuukk. Universiteit Utrecht/Purdue University, Utrecht, Netherlands.

Current neurotoxicity testing relies on animal experiments, even though they are ethically debated and require inter-species translation. These models are often not predictive for human risk, for example, when it comes to chemical-induced seizures. There is thus a clear need for an alternative. The introduction of human iPSC-technology created opportunities for animal-free seizure liability assessment. Here we aim to show the usability of commercially available hiPSC-derived neuronal co-cultures for in vitro neurotoxicity testing and seizure liability assessment. Co-cultures of glutamatergic and GABAergic neurons and astrocytes were exposed to 11 seizurogenic and non-seizurogenic compounds (PTZ, amoxapine, enoxacin, amoxicillin, linopirdine, pilocarpine, CPZ, phenytoin, PTX, 4-AP and strychnine). Acute effects on spontaneous neuronal network activity were assessed using micro-electrode array (MEA) recordings. In parallel, rat primary cortical cultures were exposed. LOECs were comparable following exposure to amoxapine (0.03 µM), linopirdine (1 µM) and pilocarpine (0.3 µM). In all other cases, hiPSC-derived neuronal co-cultures were more sensitive (LOEC (µM) hiPSC - rat cortical cultures; PTZ 30 - 10; PTX 0.03 - 0.3; strychnine 0.3 - 3; enoxacin 0.1 - 3; 4-AP 1 - 3; CPZ 0.1 - 10; phenytoin 0.3 - 3). In the case of amoxicillin, no LOEC for rat cortical cultures could be calculated, while the LOEC for hiPSC-derived neurons was 1µM. We further found that based on chemical fingerprints, spike parameters are more sensitive for excitability in rat primary cortical cultures, whereas in hiPSC-derived co-cultures, most parameters are less affected. To determine risk on a more individual level or for specific vulnerable populations, subject-derived hiPSC-derived neurons offer possibilities. An example is shown where activation of insulin receptor/insulin growth factor receptor and AKT signaling associated with manganese exposure (1 µM-500µM) is reduced in iPSC-derived neuronal progenitors of Huntington’s disease subjects versus control subjects. Overall, our data indicate that hiPSC-derived neuronal co-cultures can be used for in vitro neurotoxicity testing. Taken together with the fact subject-derived hiPSC-derived neurons can be developed, it is clear that these neurons hold great potential for the future of animal-free in vitro neurotoxicity testing.
Animal tests for Developmental Neurotoxicity (DNT) have been designed and required by regulatory authorities. However, because of complex underlying mechanisms and questionable relevance to humans, limitations of those current approaches are enormous. The stakeholders in the field are forming research committees and regularly conducting workshops to discuss the progress in development of alternatives for both toxicity testing and drug screening (e.g., OECD DNT task group and International STAND-UP NETwork (ISTNET), working on improving regulatory assessment and DNT OECD test guidelines based on new approach methodologies; micro-physiological systems or organ-on-chip consortia are focusing on identifying gaps and needs for use of such organ-on-chip technologies for pharmaceutical industry. The main goal of our research is to develop a testing strategy based on our human 3D iPSC-derived brain model, for a DNT IATA (Integrated Approaches to Testing and Assessment). We showed earlier DNT of the pesticide rotenone and the antidepressant paroxetine in this model. Our iPSC-derived BrainSphere model covers many key events of neural development, which allows assay multiplexing. Currently we are developing an assay using CRISPR/Cas9 knock-in fluorescent tags for neural markers (6-in-1 BrainSphere assay) which should increase DNT testing throughput. The iPSC used for BrainSpheres allow the study of molecular mechanisms of neurological disorders by using patient-derived or genetically modified cells. We are using Amyotrophic Lateral Sclerosis (ALS) patient-derived iPSC, to study ALS mechanism and screen drug library. To study gene environmental interactions (GxE) in autism we used CRISPR/Cas9 modified iPSC with mutation in autism risk gene CHD8. We found an interplay in mRNA and metabolic biomarkers between CHD8 mutation and the organophosphate pesticide chlorpyrifos. Taken together, iPSC-derived BrainSpheres represent a versatile tool for mechanistic understanding of diseases, studying GxE and screening drugs and chemicals.

Multiple non-animal-based test methods for neurotoxicity testing have never been formally validated. Therefore, before hiPSC-based test systems can be used they have to be evaluated following criteria to define their readiness regarding various regulatory applications. During this presentation, human iPSCs-derived test methods (models and endpoints) for neurotoxicity testing will be presented, showing different test readiness levels, depending on the intended use of in vitro data. Readiness criteria which were compiled during a stakeholder workshop (by academia, industry and regulatory authorities) will be characterized referring to test systems, exposure schemes, main measured endpoints, tools for cytotoxicity prediction, test data, reproducibility, test benchmarks, prediction model, applicability domains and definition of screening hits. The evaluated in vitro assays are anchored to key neurodevelopmental processes that are essential for nervous system development including cell migration, proliferation, neurite outgrowth, synaptogenesis and neuronal network formation and function. It is assumed that neurotoxicants exert toxicity by disturbing at least one of these processes. Based on the (semi)-quantitative analysis, readiness of 17 in vitro assays for neurotoxicity testing will be discussed with respect to various regulatory uses (e.g. prioritization/screening, hazard identification/characterization and risk assessment). The testing state of these assays was compiled for more than 1000 compounds. The scoring results suggest that several assays are currently at high readiness levels, especially for screening purposes since the acceptable level of uncertainty can be higher when compared to hazard or risk assessment. It is advisable that a battery of in vitro DNT test methods is based on complex mixed neuronal/glia cultures derived from human iPSCs in order to avoid extrapolation of results and to be as close as possible to human biology. During this talk, suggestions will be made on how in vitro test methods may be assembled into testing strategies for various regulatory purposes. Finally, a vision will be presented on how further development of new in vitro methods may be guided by knowledge of signaling pathways necessary for brain function as well as those involved in pathophysiology and relevant adverse outcome pathways.

Drug-induced liver injury (DILI) and hepatobiliary diseases remain significant issues for both drug developers and clinicians seeking to improve and advance the health of patients safely. Bile acids (BA) have been recognized as both indicators and causative agents of various liver toxicities for many years, but only with recent technological advances has the true potential of BA as biomarkers become clear. However, many significant obstacles currently exist, including the sensitivity and specificity of a BA response in a disease state or to a therapeutic agent, marked species variability in bile acid profiles, and an incomplete understanding of the factors controlling BA homeostasis. Similarly, the sheer amount of data generated leads to technical and interpretative challenges that arise due to the complexity of the integrated analysis required for quantification of individual BAs. Despite these unknowns, the potential of BA to provide additional insight into hepatobiliary disease has generated significant excitement across the field. This workshop features three speakers who will focus on developments in understanding the utilization of BA in clinical diagnosis and drug development. Included among the discussion topics are (1) the use of BA indices and a score model to simplify a complex dataset to predict mortality and progression of cholestatic liver disease; (2) a real-world case study of how perturbing bile acid homeostasis can impact drug development; and (3) how establishing reference ranges for individual bile acids (IBA) can lead to improved clinical diagnoses of hepatobiliary disease. This discussion will conclude with an interactive panel discussion driven by attendee questions. Together, these talks will highlight the challenges, opportunities, and application of bile acid profiling to further both the mechanistic understanding and the diagnosis of hepatobiliary injury.

Hepatobiliary diseases result in the accumulation of toxic bile acids (BA) in the liver, systemic blood, and other tissues leading to an unfavorable prognosis. Despite the extensive efforts, the use of BA as biomarker for liver diseases has not translated into clinical practice primarily due to limitations including the differences of the physiologic and pathologic effects of the various individual BA, as well as the extremely high inter- and intra-individual variability of BA concentrations. These limitations could be addressed using “BA Indices”, which are ratios calculated from the absolute concentration of individual BA and their metabolites. These ratios quantify in detail, the composition, hydrophilicity, metabolism, formation of secondary BAs, and toxicity of the BA profile. BA indices have markedly low intra- and inter-individual variability and are resistant to food consumption, age, gender, BMI, etc. BA indices outperformed serum liver enzymes such as ALT and AST as biomarkers for the diagnosis of cholestatic liver diseases. Furthermore, we developed the bile acid score (BAS) model, a survival model based on BA indices, to predict the
Drug-induced liver injury (DILI) is a leading cause of drug development failures, and it often arises from a complicated etiology. Susceptibility factors that increase the risk for DILI have been identified, can be assessed in vitro, and include mitotoxicity, cytotoxicity, and inhibition of key bile acid (BA) transporters. To date, these assays are used for hazard identification; however, there is increasing recognition of their utility for prospective risk assessment and compound triage. Overall, the contributions of BA handling to DILI are poorly understood; therefore, the clinical translation is uncertain. Notably, one challenge is BA transporter inhibition, which hypothetically leads to accumulation of toxic bile acids and xenobiotics inside the hepatocyte and thus hepatocellular injury. There also is increasing information about BA effects on small and large cholangiocytes in health and disease. Increases in total BA levels in the blood are often associated with cholestatic injury but emerging data suggests this could be an oversimplification based on the current diagnostic paradigm. Due to pronounced species differences in BA metabolism, DILI related to a bile acid-related specific mechanism is difficult to detect with routine nonclinical toxicity studies, thus underpredicting or falsely predicting human risk. BA transporter inhibition is thought to perturb BA homeostasis that can be quantified by assessing BA blood levels. With the advent of sophisticated methods for separation and quantification of over 50 individual bile acids, this presentation will address the translational value in assessing BA handling from early in vitro assays through clinical trials. We will also demonstrate the complexity of the cross-talk and modulation of interconnected pathways that ultimately influence the BA profile. These include pathways related not only to bile salt transporters, but also hormone transporter receptors, local phospholipid and arachidonic acids, CYP enzymes, bile acids, and sulfation. As transaminase enzymes are sensitive and relatively specific biomarkers for DILI, it will be important to start to define the overall value and the context-of-use of adding individual BA to the toolbox to better assess DILI risk and mechanisms.
1254 Transcriptome Points of Departure: Derivation Methodology, In Vivo Stability, and Concordance to In Vivo Short-Term Apical Points of Departure

K. Johnson. Corteva Agriscience, Indianapolis, IN.

This presentation will describe methods to generate transcriptome points of departure and examine their temporal stability and concordance to short-term study apical POD values. Developing a consensus method to derive a molecular POD will be crucial to use the methodology within a regulatory framework; however, no consensus exists currently. Two methods to derive a transcriptional POD will be presented. The first method is based upon the historical practice of deriving a gene-based (e.g., Gene Ontology terms) POD using the Functional Classification step with BMDExpress software. The second method to be presented will explore removing the Functional Classification step and instead utilizing POD values at the level of individual genes. Both of these methods are agnostic to mechanism in that a direct link- age between the gene set or individual genes driving the POD and the apical outcome is not obtained. In effect, the transcriptional POD may represent the first point along the dose response curve of “concerted molecular change”. Using rat liver TG-GATES transcriptome data across 79 molecules and eight time points, the stability of the transcriptome POD over various durations of exposure will be presented. Finally, concordance of the rat liver transcriptional POD to a short-term (29 day) rat “systemic” apical POD will be shown using data across 79 TG-GATES molecules. This presentation will highlight the conclusion that a rat transcriptional POD agnostic to mechanism or mode-of-action is 1) robust to exposure duration and 2) predictive of an in vivo apical POD. This information sets the stage for the subsequent presentations exploring additional in vitro and in vivo case studies comparing the predictivity of a transcriptional (or high content imaging-based) POD for an apical POD and outlining the potential use of molecular PODs in regulatory contexts.

1255 In Vitro Molecular PODs from High-Throughput Profiling Assays

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

US EPA has been exploring the use of New Approach Methodologies (NAMs) for hazard characterization of environmental chemicals for more than a decade. In order to increase screening efficiency and coverage of human biological space in the context of in vitro hazard characterization, the recently released Blueprint for Computational Toxicology at US EPA advocates the use of high-throughput profiling (HTP) assays as the first step in a NAMs-based tiered testing framework for hazard characterization. Ideally, HTP assays should be capable of being deployed in high-throughput screening format across multiple human-derived in vitro models and provide high content data that can be used to assess perturbation of intact biological networks and/or cellular functions and inform putative mechanism-of-action prediction. To date, US EPA has identified two assays that satisfy these criteria: 1) high-throughput transcriptomics (HTTr) using TempOLigo-seq and 2) high-throughput phenotypic profiling (HTPP) using Cell Painting. Information gleaned from these HTP assays include molecular point-of-departures (PODs) based on threshold concentrations for perturbation of cellular biology. These molecular PODs may either be agnostic to chemical mechanism or anchored to established key events or mechanisms-of-action based on prior knowledge or inference from the observed changes in gene expression or cellular morphology. A brief overview of each methodology will be presented along with demonstration of how data from each of these assays can be used to generate molecular PODs through the use of high-throughput concentration response modeling and conversion to administered equivalent doses (AEDs) using high-throughput toxicokinetic modeling and reverse dosimetry. Molecular PODs derived from the HTP assays will be compared across technologies and across cell lines as well as to molecular PODs that can be derived from “traditional” HTS assays within the ToxCast / Tox21 assay suite. Lastly, the utility of the molecular PODs for chemical safety assessment will be demonstrated in a screening for prioritization context using bioactivity:exposure ratio (BER) analysis. Participants in the session will gain a broader understanding of emerging high-throughput technologies and their potential applications for NAMs-based chemical safety assessment. This abstract does not reflect US EPA policy.

1256 Case Studies on the Use of Transcriptome Points of Departure

C. Yauk. University of Ottawa, Ottawa, ON, Canada. Sponsor: J. Rager

Case studies provide important opportunities for the regulatory and research communities to work together to define context of use and advance new approach methodologies. Benchmark-dose modeling of transcriptomic data has numerous potential applications in regulatory decision-making, from tiered testing/prioritization to derivation of a molecular-based point of departure (mPOD) for use in risk assessment. Exemplary case studies on different applications and approaches will be presented. Perspectives will be given on lessons learned from case studies on the flame retardant hexabromocyclododecane in which a tiered testing approach was employed and a set of perfluorinated chemicals in which transcriptomics was used to characterize relative potency and derive bioactivity exposure ratios. A point of emphasis will be placed on illustrating the impact that the use of the in vitro and in vivo transcriptomic approaches has on determining a mPOD and how these compare to PODs based on guideline toxicological assessment data (i.e., a pathology based POD). The case studies will provide context for understanding the applicability of mPODs and the implications for using them for risk assessment.

1257 Quantitative Integration of NAMs into Regulatory Decision-Making: Current State-of-the-Science and a Michaelis-Menten-Based Acceptance Path Forward

J. Lambert. US EPA, Cincinnati, OH.

The vast majority of chemicals found in commerce and the environment are data-poor chemicals, and as such are commonly unaccounted for in formal quantitative evaluations of health risks to human populations. This is due to the lack of available points-of-departure (PODs). The utility of the rat liver transcriptional POD to a short-term (29 day) rat “systemic” apical POD will be shown using data across 79 TG-GATES molecules. This presentation will highlight the conclusion that a rat transcriptional POD agnostic to mechanism or mode-of-action is 1) robust to exposure duration and 2) predictive of an in vivo apical POD. This information sets the stage for the subsequent presentations exploring additional in vitro and in vivo case studies comparing the predictivity of a transcriptional (or high content imaging-based) POD for an apical POD and outlining the potential use of molecular PODs in regulatory contexts.

1258 Paving the Way for Greater Data Sharing to Advance Biomarker and Drug Development: Industry, Academia, and Regulatory Insights

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Data is at the heart of scientific advancement and biomarker and translational model development. Combining complementary strategies and pooling scientific data through cross-industry, academia, and regulatory collaborations with increased data sharing would bring unprecedented power, insight, and game-changing solutions to biomarker and translational model development (e.g., predictive safety and efficacy models). Utilizing existing data is both more efficient and more ethical. Yet, data sharing is still challenging. Pre-competitive collaborations, crowdsourcing, and open science sourcing (i.e., diverse strategies that seek external input and public engagement) can harness the power of data sharing using newly developed tools that protect proprietary data. These efforts will foster new and improved drug discovery and development to enable more effective and safer therapies. This Workshop will highlight ideas, ongoing efforts, and future aspirations from industry, consor- tium, academia, and the US FDA on data sharing and how increased collabora- tion and new ways of working together can have greater impact on novel biomarker development and the drug discovery and development process. Data will be shared from a cross-industry survey conducted by the IQ DruSafe Biomarker Working Group on the current and future state of emerging safety biomarkers and will include case examples from data collected as part of the survey. An emphasis will be placed on what is needed to modernize the development of methods, materials, and measures that can streamline drug development. This will include new data sharing tools being developed by the Critical Path Institute’s (C-Path) Predictive Safety Testing Consortium (PSTC) and Translational Quantitative Safety Testing Consortium (TransQST) to protect proprietary data and bring new medicines to patients in a more efficient manner. Case studies and examples will be shared and will include collaborative efforts presented by the US FDA showcasing the power of data sharing on patients. The Workshop will finish with an interactive panel discussion to foster open communication and dialogue with Workshop participants.
The importance of scientific data sharing has been recognized in drug discovery and development for more than a decade. Pre-competitive consortia and academic and agency collaborations have been created to address the need for medical advances including biomarker discovery and development and more translation models of efficacy and safety that rely on data and information sharing. The consortia differ in their goals, members, operations, and outputs, but all are based on the understanding that advancing human health takes a village. This introductory talk will provide a brief overview of the current collaborative landscape and how paving the way forward through increased data sharing will help advance biomarker development and the drug discovery and development process to bring more effective and safer medicines to patients.

Biomarkers have become integral to the success of drug development. Given that safety concerns remain an important cause of drug attrition, there is a significant unmet need for successful implementation of emerging safety biomarkers (ESBs) that can predict, diagnose, monitor, or characterize drug toxicities. Successful ESB implementation holds tremendous potential to accelerate drug development (DD) but still face many obstacles. For example, the use of ESBs in decision-making is often undisclosed so that their broad impact in drug development (end-to-end) remains unclear. Pre-competitive organizations that foster information- and data-sharing provide opportunities to reveal the current and future states of safety biomarkers. To that end, the IQ DrugSafe Safety Biomarker Working Group with representatives from 16-member companies conducted a survey of 20 pharmaceutical and biotech companies to assess challenges and opportunities for the current and future state of ESBs found in biofluids (protein-, activity-, metabolites-, and mRNA/mRNA- biomarkers but not tissue-based biomarkers). Questions were designed to 1) determine current use of ESBs in the nonclinical/clinical space and the impact on asset advancement in drug development; 2) identify opportunities, gaps, or barriers to greater implementation of ESBs; 3) assess ESB impact on advancement of DD; and 4) benchmark perspectives on regulatory acceptance of ESB. The results from the survey will be highlighted with an emphasis on the Future State and what is needed moving forward to enable use and broader application of ESBs in both nonclinical and clinical phases of DD including a need for: 1) more robust science balanced with practical approaches to evaluate strengths and weaknesses of ESBs; 2) increased cross-industry collaborations to develop well-validated assays and decrease the time and resources required for ESB development; and 3) clear written criteria by Health Authorities regarding detailed requirements for increased ESB biomarker acceptance. Use-case examples focusing on ESBs in nonclinical and clinical settings will be highlighted. *Authors listed represent all contributing authors from the DrugSafe Emerging Safety Biomarkers in Nonclinical and Clinical Studies Focus Group of the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ).
has been a serious debate about how to treat fluoropolymers. This topic was addressed in the FTM, in active EU-based regulatory proposals and in other media. This SOT discussion will tackle head-on these classification issues, provide an update on current thinking and focus on polygons of low concern. To close, many of the above topics will be summarized by the PFAS experts that participated in the Durham 2019 FTM in several upcoming peer-reviewed publications.

1267 PFAS Ecotoxicology
G. Ankley, US EPA, Duluth, MN. Sponsor: M. Carvan
Per- and poly-fluoroalkyl substances (PFAS) have achieved substantial prominence in terms of public visibility and regulatory concern. Research and regulatory activities associated with the latest with PFAS in many parts of the world have emphasized possible human health effects, but there is increasing concern for potential ecological effects of the chemicals. For example, some PFAS have been listed as persistent organic pollutants (POPS) under the Stockholm Convention based on both potential human health and ecosystem considerations. A major challenge for scientists and risk assessors assessing the ecological risks of PFAS is the substantial number of compounds that may need to be considered in terms of potential accumulation and effects on a variety of aquatic and terrestrial species, often with little or no fate or toxicity data. Requirements to support ecological risk assessment of PFAS include research to support the formulation of predictive models for bioaccumulation, and the development of cost-effective in silico, in vitro, and in vivo methods to rapidly assess biological effects for potentially sensitive species/endpoints. Addressing needs associated with assessing the ecological risk of PFAS will require cross-disciplinary approaches that employ both conventional and new methods in an integrated, resource-effective manner. This abstract does not necessarily reflect the opinion of the US EPA.

1268 PFAS Human Health
C. Ng, University of Pittsburgh, Pittsburgh, PA.
New in vitro and in silico models are being sought and developed to aid with better anticipating risks associated exposure to per- and polyfluoroalkyl substances (PFAS), ubiquitous and persistent environmental contaminants. Given the large number of identified PFAS, a substance-by-substance approach is simply too slow and resource-intensive. Three complementary approaches were developed in our group to predict the biological fate and effects of PFAS: molecular, toxicokinetic, and machine-learning-based classification models. We will highlight a recently completed molecular docking study of PFAS interactions with the major BBB efflux transporter, P-glycoprotein (P-gp). Comparing binding sites and affinities for a variety of legacy and emerging PFAS with known P-gp inhibitors and substrates, we found first- and second-generation inhibitors have the lowest binding affinities and long-chain PFAS the highest. Using clustering analysis, we found PFOA and PFOS are clustered separately from all other ligands, indicating their inhibition mechanisms may be unique. Understanding such interactions provides critical insight to parameterize toxicokinetic models to predict tissue distribution and half-lives. As we demonstrate with a model for PFOA in the male rat, parameterization using only in vitro PFAS data can predict tissue distribution and elimination kinetics within a factor of 2 of in vivo data. Thus, model performance is as good as traditional approaches that rely on fitting to in vivo data. However, for PFAS with almost no in vitro or in vivo data available, parameterization of detailed mechanistic models can be difficult. For such “untested PFAS” we developed a machine learning method to classify 3,486 PFAS in the OECD database, identifying 26 as yet untested PFAS with high rates of bioactivity across multiple assays. These complementary in vitro and in silico strategies can guide prioritization and inform high-throughput assessments to prevent continued exposure to toxic chemicals and instances of regrettable substitution. The approaches presented here provide a platform for integrating multiple lines of evidence for increasing confidence in these new approach methodologies to predict PFAS fate and effects across species.

1269 Safety Assessment of Devices Used in Assisted Reproduction Technology: Mouse Embryo Assay
N. S. Goud, Greenwood Toxicology Associates LLC, Greenwood, IN.
Medical devices are being increasingly used in the diagnosis and treatment of various conditions. One such example is the devices used in assisted reproduction technology (ART) such as catheters, needles, and culture dishes and media. The success rate of in vitro fertilization (IVF) depends not only on the
nature of sperm and eggs obtained from the couples but also on the quality of devices used in the process. Before such devices are marketed for clinical use, care should be taken to ensure that they meet regulatory requirements for patient safety (in this case, to the survival of embryo/fetus). Though there are existing standards by ISO 10993 series, ASTM, and USP on the manufacturing and biocompatibility testing of different medical devices, unfortunately there were no requirements until recently for ART device category. Since ART devices contact (either directly/indirectly) the gametes (sperm/eggs) and/or embryo, testing them in the mouse embryo assay (MEA) is considered relevant. The first speaker, from the US FDA, will tell the history of the development of the MEA guidance document introduced in June 2019. He will describe the salient features of embryo culture and how the devices are tested. Particular focus will be on parameters of mouse strains, number of embryos for test and control groups, sample size, extraction methods, culture conditions, and acceptance criteria, which are essential for successful premarket submission to US FDA. Currently, IVF is a method of choice in the treatment of infertility. As per the recent CDC report, approximately 1.9% of all infants born in the United States every year are conceived using ART. The second speaker will give his perspective on the MEA as a functional and toxicological bioassay in detecting embryo toxicity; the standard procedures of using F1 hybrid mice in the development of one-cell or two-cell embryos and the acceptance criteria of percent blastocyst development; and methods in assessing the morphology and viability of embryos and the role of oil and ingredients in media such as protein and volatile organic chemicals on fertilization. Some devices are tested directly in the MEA, but extracts can also be used. He also will explain how various ART devices that are evaluated get market approvals not only for US FDA but also for other global regulatory bodies. The last speaker will describe the effect of detergents and cleaning agents used during the manufacturing of ART devices and the role of coatings/adhesives and endotoxins on the process of fertilization in some case studies. Perfumes and antiperspirants used by prospective parents, workers in device manufacturing plants, or health care staff in reproductive clinics also have an impact on embryo viability. In summary, the purpose of this informational session is to highlight the importance of testing the ART devices to the newly developed US FDA guidance on the MEA to minimize embryo lethality, which in turn could bring cheers to prospective parents and family.

**1270 Application of Computational Genomic Approaches to Address Toxicity Mechanisms and Prediction**

M. Gosink, Pfizer Inc., Groton, CT.

The physiochemical properties of compounds contribute to their safety and have been extensively used to predict toxicity. However, genomic context also plays a significant role in the development of compound-induced toxicity. Genomic information can provide critical insight into mechanisms and help improve the prediction of relevance of toxicities to humans and/or identify specific subpopulations that can avoid liability. This session will focus on the utilization of computational genomic approaches and information to elucidate toxicity through off- and on-target mechanisms and how genomic information can then be utilized to predict toxicity. The first two talks will focus on the utilization of coding and non-coding RNA expression data to understand toxicity mechanisms. The third talk will also use expression data but will delve into prediction of drug on- and off-targets. The fourth speaker will present on how individual genomic context can affect drug response and how this pharmacogenomic information is used by the US FDA in their evaluation of pharmaceuticals. Finally, the last presenter will discuss the work done by an academic/government/industry collaboration to develop a transcriptomic biomarker of genotoxicity and their efforts to get it accepted by the US FDA.

**1271 Analyzing Gene Expression Data to Elucidate Toxicity Mechanisms**

R. Grafröström, Karolinska Institutet, Solna, Sweden.

“Toxicogenomics” represents a steadily developing “Big Data” informatics analysis field. Increasing amounts of safety testing-derived gene expression data require related to device and workflow. This data is critical to elucidating toxicity mechanisms. To interpret the vast amount of available information contained within gene expression data, our laboratories utilize a tiered prioritizing approach for analyzing high-throughput (HT) screening-derived cytotoxicity and omics results in cell culture models. The cost-effective approach allows libraries of agents to be hazard ranked to the mode-of-action (MoA) level. Further, a data-driven 14 gene component-based “predictive toxicogenomics space (PTGS)” tool provides toxicity estimates intrinsic to omics-data via broad coverage of toxicity reactions and mechanisms. This tool further enables application of in vitro data to assess tissue injury in multiple organs of experimental animals subjected to repeated-dose toxicity bioassays, including the accurate prediction of human drug-induced liver injury. The PTGS component modeling concept may be the first high-throughput analysis tool that effectively bridges systems biology/systems toxicology and AOP-directed linear data integration by defining toxic MoA coupled with key events in AOP schemas.

**1272 Computational Prediction and Wet-Lab Validation of Non-coding RNAs in the Regulation of Drug Metabolizing Enzymes Related to Drug Efficacy and Safety**

B. Ning, US FDA/NCTR, Jefferson, AR.

Drug metabolizing enzymes and transporters (DMETs) mediate biotransformation of drugs and play an essential role in drug efficacy and toxicity. We conducted studies to characterize mechanisms involved in noncoding RNAs (ncRNAs) mediated regulation of DMETs, using in silico, in vitro and in vivo approaches. First, using the expression patterns of ncRNAs and miRNAs to elucidate the relationships between ncRNAs and mRNAs is a powerful in silico approach. Many databases contribute wealth resources for in silico analyses of gene expression patterns to examine potential off-target candidates and their target mRNA molecules. Second, publicly available prediction tools were used to predict the interaction between ncRNAs and mRNAs. However, it is common for a prediction algorithm to generate many false positive candidates, since the prediction theory is primarily based on a base-pairing between the seed of an ncRNA and its target genes with different stringencies in utilizing predictive parameters. We improved the confidence in the predicted result by comparing predictions using different algorithms. Third, the calculation of a minimum free energy of hybridization (∆G) between an ncRNA and its cognate mRNA is another in silico method to predict the affinity of an ncRNA-mRNA interaction. We created a fluorescence-based in vitro electrophoretic mobility shift assay to visualize the interaction between an ncRNA and its target mRNA, which provides direct evidence of ncRNA-mRNA interactions. To validate computational prediction, we used wet-lab strategies including reporter gene assays, electrophoretic mobility shift assays, enzymatic assays, toxicological assays to characterize the specificity and potency of ncRNA-dependent molecular mechanisms in regulating the expression of DMETs such as CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, ALDH5A1, SULT1A1, SULT2A1, ABCB6, ABCB3 and SLCO2A1. Furthermore, we showed that interactions between ncRNAs and drugs can influence drug-drug interactions and drug toxicity. Notably, our studies demonstrated that the integration of computational prediction and wet-lab validation is an important approach to elucidate mechanisms of ncRNAs in the regulation of DMETs related to drug efficacy and safety.

**1273 Exploring the Use of Compound-Induced Transcriptomic Data Generated from Cell Lines to Predict Compound Activity toward Molecular Targets**


Pharmaceuticals or phytopharmaceuticals molecules rely on the interaction with one or few specific molecular targets to induce their anticipated biological responses. Nonetheless, these compounds also are prone to interact with many other biological targets, also known as off-targets. This fact is particularly critical when some (or a combination of) off-targets are associated with adverse effects. Unfortunately, off-target identification is extremely difficult and expensive. Consequently, QSAR models predicting the activity of a molecule on a particular off-target have gained importance in the de-risking process of pharmaceuticals. For example, such models can be trained to predict the risk associated with mutagenicity, oral acute toxicity, or endocrine related toxicity. However, few off-targets are well characterized with enough data to build such in silico models. A good alternative to individual off-target evaluations is the use of integrative evaluations such as transcriptomics obtained from compound-induced gene expression derived from in vitro cell cultures. The advantage of this strategy is the capability to determine the consequences of the interaction of compounds on many possible molecular targets and biological pathways, without having any constraints concerning the chemical space. In this work, we assess the value of compound-induced transcriptomic data to build machine-learning models that can predict molecular targets (off-targets) causally associated to a known toxicity. For this, we trained random forest models using data from the cMAP L1000 dataset, which contains a large number of compound-induced gene expression pro-
files. Transcriptomic-based machine learning models were able to predict off-targets using data generated from appropriate cell lines; even in some cases, outperforming QSAR models. We also provide a simple framework to determine in which cases the use of transcriptomics data exploring biological spaces can help to overcome the limitations derived from a restricted chemical space.

# 1274 The Role of Pharmacogenomics in Understanding Adverse Drug Reactions


Adverse drug reactions (ADRs) continue to be a source of concern for early and late phase clinical development, with drug induced liver injury being the most notable, although others such as cutaneous adverse reactions and nephrotoxicity also arise. During the past decade increased use of genetic sequencing has deepened our understanding of the potential role of genetic variants in predisposing patients to ADRs. The most common associations are found with the Human Leukocyte Antigen (HLA) genes, where variants such as HLA-B*15:01 have previously been found to be associated with Stevens-Johnsons syndrome, such that the FDA recommends that patients with that variant do not use Abacavir. There are currently 51 such recommendations by FDA on using pharmacogenomics to avoid either toxicity or ineffective use of a drug if a patient has a specific genetic variant. This talk will focus on the current understanding of pharmacogenomics and ADRs and use in drug development.

# 1275 Utility of TGx-DDI Biomarker for Genotoxicity Hazard Assessment of Pharmaceuticals and Environmental Chemicals


Genotoxicity testing is an essential component of the safety assessment paradigm required by regulatory agencies world-wide for analysis of drug candidates, and environmental and industrial chemicals. Current genotoxicity testing batteries feature a high incidence of irrelevant positive findings in the in vitro chromosome damage tests. These irrelevant positive findings pose a major challenge for risk management and therefore require complex, time consuming, and costly follow-up strategies, including animal testing. Thus, there is an urgent need to develop new testing approaches for regulatory decision making. Using machine learning, we identified a transcriptomic biomarker named TGx-DDI that consists of transcripts that responds to DNA-damage in human cells and proposed context of use to improve genetic safety assessment for environmental chemicals and pharmaceuticals. We validated the performance of the TGx-DDI biomarker to identify true DNA damaging agents using a large set of chemicals, assessed intra- and inter-laboratory reproducibility, and cross-platform performance. In addition, we also developed a high-throughput version of TGx-DDI that enable large scale testing and prioritization of pharmaceutical lead compounds, environmental and/or industrial chemicals for further testing. To enable TGx-DDI use in regulatory decision making we are pursuing a formal biomarker qualification under the FDA biomarker qualification program. This presentation will summarize the development of TGx-DDI in context of biomarker qualification process and provide several case studies that demonstrate its utility in an integrated genotoxicity testing paradigm for drug candidates and environmental chemicals.

# 1276 Botanical Mixtures: Predictive Approaches to Evaluating Pregnancy, and Reproductive and Developmental Health

M. Huang, NIEHS/NTP, Research Triangle Park, NC.

Botanical dietary supplements are widely used throughout the world. They have long had a place in preparing for or alleviating pain from pregnancy, easing nausea and vomiting, treating urinary tract infections, and facilitating labor. After birth, they have been used to initiate, maintain, and/or augment milk production during lactation. Reports from both Australia and the United States indicate that 36% and 45% of respondents, respectively, have used at least one herbal product during pregnancy. While women in particular use herbal products, there also is a wide array of botanicals available for men (e.g., to enhance sexual function) and children (e.g., to improve behavior and or development). During the past decade, increased use of genetic sequencing has deepened our understanding of the potential role of genetic variants in predisposing patients to adverse drug reactions. The most common associations are found with the Human Leukocyte Antigen (HLA) genes, where variants such as HLA-B*15:01 have previously been found to be associated with Stevens-Johnsons syndrome, such that the FDA recommends that patients with that variant do not use Abacavir. There are currently 51 such recommendations by FDA on using pharmacogenomics to avoid either toxicity or ineffective use of a drug if a patient has a specific genetic variant. This talk will focus on the current understanding of pharmacogenomics and ADRs and use in drug development.

# 1277 An Overview of Botanical Dietary Supplements: Key Research Needs and Unique Challenges of Botanical Research

M. Huang. NIEHS/NTP, Research Triangle Park, NC.

Botanical dietary supplements are products made from plants, either in whole, in part, or as an extract, that are meant to be consumed to supplement the diet. Due in part to the regulation of botanicals as dietary supplements, there is a plethora of these kinds of supplements available to consumers. The efficacy of many of these supplements remains untested and there is a general perception of botanical dietary supplements as “natural” and thus presenting minimal harm. However, there is little safety data available to support this assumption. Given the widespread use of botanicals by women, many who are pregnant or of child-bearing age, understanding potential effects of botanicals on reproduction and development is of high importance for women’s and children’s health. This talk will introduce the field of botanical dietary supplements, what they are, what they are used for, and the extent of their usage. Next, it will expand on some of the unique challenges associated with studying botanicals, such as inherent test article variation, contamination vs. adulteration, evaluating botanicals as mixtures, and active ingredient characterization. This presentation will also highlight the particular importance of understanding reproductive and developmental toxicity for these compounds and discuss a case study of black cohosh, work that has been done at the National Toxicology Program. Overall, this presentation will set the stage for a discussion of various approaches to tackling the challenge of botanical research in the context of developmental and reproductive toxicity.

# 1278 Global Regulations for Botanical Supplements and Their Role in Risk Mitigation of Phytochemicals in Breast Milk

R. J. Marles. Health Canada, Ottawa, ON, Canada. Sponsor: M. Huang

The U.S. and other jurisdictions regulate botanical supplements as medicines, dietary supplements, food supplements or supplemented foods – in many cases the same botanical ingredient may fall into multiple categories within a single jurisdiction. Basketing into one or more categories depends on the legislative and regulatory frameworks in force. Key product classification criteria include product format (dosage form vs. food form); recommended conditions and directions for use (explicit claims, serving size, daily intake); composition (e.g., ingredients in regulatory schedules, toxicity); representation (packaging, labeling, advertising, implied claims); public perception and history of use (e.g., cultural practices). How a product is regulated affects how considering the possibility of exposure during pregnancy. The precautionary principle would state that herbal medicines and supplements should not be taken during pregnancy or breastfeeding unless the benefit to the mother outweighs any possible risk to the fetus/nursing infant. While this recognition may serve as general medical advice, there are clearly instances, as described, where pregnant/breastfeeding women are using botanical preparations under the direction or at the advice of health care providers, without oversight. These issues are of concern, as some women may not even be aware that they are pregnant in the early stages of pregnancy. Labeling alone may not be the most appropriate or effective risk mitigation measure to warn women not to use herbal supplements during pregnancy. There is a need for evidence-based safety data that include evaluation of developmental and reproductive toxicity (DART) endpoints. Research on these botanical dietary supplements is complicated by the many diverse botanical products marketed for reproductive health, their chemical complexity due to the fact that many are mixtures, inherent variations in different extracts used in different products, and a lack of understanding of the biologically adverse effects or of ability to identify long-term reproductive and/or developmental consequences. In this session, speakers will address the current procedures/methodologies for assessing botanicals in DART studies, the global regulatory guidelines, efforts to improve or design new methodologies, and successes/challenges associated with such approaches. The session will begin with an overview of botanical dietary supplements, highlighting the importance of evaluating DART for these complex mixtures and the unique challenges associated with studying them. Next, there will be a presentation of the regulatory landscape surrounding botanical dietary supplements in various regions around the world and the approaches being used and developed to inform risk assessment. Following speakers will present select examples of in silico and in vitro approaches for botanical ingredients and the successes and issues associated with these new testing paradigms. This session will highlight ongoing efforts to address challenges of generating safety data regarding botanical use during sensitive life stages and potential methodologies and challenges and will provide suggestions for paths forward for addressing botanical mixtures research.
consumers use it and what tools are available to mitigate risks (e.g., recalls, adverse reaction reporting and causality assessment, public warnings). The international range of frameworks for botanicals will be illustrated by considering the types and sources of phytochemicals reported to have been detected in human breastmilk and the available tools for risk mitigation.

**1279 The Botanical Safety Consortium’s Developmental and Reproductive Toxicity Technical Working Group**

C. A. Mitchell. HESI, Washington, DC. Sponsor: M. Huang

The Botanical Safety Consortium (BSC) is a public-private partnership that embodies the spirit of applying predictive toxicological tools to the evaluation of botanical ingredient safety. The BSC is a collaboration between scientists in industry, government, and academia, formed with the goal of providing a sound scientific basis for integrating existing data with the latest toxicology tools to evaluate botanical safety. Chemical characterization of complex botanical ingredients and identification of fit-for-purpose assays for evaluating developmental and reproductive toxicity (DART), genotoxicity, hepatoxicity, cardioxicity, and repeat-dose systemic toxicity are key areas to be explored by the consortium. The BSC’s DART Technical Working Group (TWG) is developing a pragmatic fit-for-purpose testing strategy for botanical ingredients to generate relevant DART data. Botanical ingredients have been selected based on reported toxicity or safety with respect to DART from case studies, animal data, or alternative methodologies. Information gathered on these selected botanical ingredients will be developed into literature reviews. The BSC’s Chemical Analysis and Data Analysis TWGs will support the DART TWG in the characterization of botanical ingredients and in experimental design. A testing strategy will be developed, including the use of high-throughput in silico and in vitro screening technologies to evaluate the potential for adverse developmental or reproductive effects. Botanicals will be evaluated as complex mixtures in these fit-for-purpose assays. These data will be made publicly available via publications and ultimately leveraged into a framework for evaluation of botanical ingredients.

**1280 Botanical Dietary Supplements and DART Screening Strategies for Decision-Making**

C. Mahony. Procter & Gamble, Egham, United Kingdom. Sponsor: M. Huang

As complex mixtures, botanicals present unique challenges when assessing safe use, particularly when endpoint gaps exist that cannot be fully resolved by existing toxicological literature. Here we explore in vitro gene expression as well receptor binding and enzyme activity as alternative assays to inform on developmental and reproductive toxicity (DART) relevant modes of action and how they can be applied to DART assessment of botanicals. Specifically, botanicals suspected to have DART effects in addition to those with a significant history of use were tested in these assays. Gene expression changes in a number of different cell types were analysed using the connectivity mapping approach (CMap), to identify modes of action through a functional read-across approach. Taken together with data obtained using a set of molecular targets customised towards known DART-relevant modes of action it was possible to inform DART risk using functional analogues, potency comparisons and a margin of internal exposure approach. The in vitro testing approaches highlighted here may also have use as predictive DART methods in areas beyond botanical safety assessment.

**1281 Challenges, Opportunities, and Solutions for In Silico Toxicology Applied to the Evaluation of DART Endpoints to Botanicals**

M. T. Cronin. Liverpool John Moores University, Liverpool, United Kingdom. Sponsor: M. Huang

In silico toxicological techniques hold the possibility of increasing the efficiency of safety assessment. They are mainly used for single, well-defined, chemicals with various methods such as (Q)SAR and read-across becoming widespread. Their application to botanicals raises a significant number of challenges. The nature of botanicals means they are not appropriate for classical in silico methods, i.e., they constitute mixtures often very complex in nature and even variable between batches and suppliers. Often, the exact constitution of a botanical mixture is not entirely known or may be poorly recorded. In addition, the mixture must be considered to include pre- and pro-haptens requiring metabolism. The fourth speaker will provide initial data for a novel dendritic cell activation assay, the THP-1 Activation Assay, which is being developed to expand the applicability domain of the current in vitro dendritic cell activation assays to include drugs that exhibit limited or no genotoxicity. The final speaker will introduce the state of the art for novel in vitro approaches based on transcriptional profiling and machine learning to predict sensitization potential. These assays are showing promise for addressing issues of difficult-to-test substances including hydrophobicity, metabolism, solubility, and formulation, and the GARDskin assay will be used to illustrate the progress. Overall, this Workshop aims to better define the applicability domains for nonanimal sensitization testing and identify progress and opportunities for expanding these domains. This Workshop represents an international collaboration between the Immunotoxicology Specialty Section of SOT and the Immunotoxicology and Chemical Allergy Specialty Section of EUROTOX in an effort to communicate and improve the science of alternative approaches for assessing potential for skin sensitization.

**1282 Applicability Domains and Future of Nonanimal Tests for Skin Sensitization**

V. J. Johnson. Burleson Research Technologies, Morrisville, NC.

Allergic contact dermatitis is an undesired side effect observed with many products, including cosmetics, natural extracts, drugs, chemicals, and medical devices. Over the last decades, a great deal of progress has been made in the development of alternative in vitro testing strategies to assess these issues, concurrent with the mechanistic understanding provided by the adverse outcome pathway (AOP) framework. The use of animals in toxicology is under ever-increasing scrutiny, with mounting pressure to develop effective alternatives. Efforts should be devoted to developing reliable in vitro assays and integrated testing strategies capable of addressing toxicity concerns for a broad spectrum of products and chemicals. This will require a better understanding of the applicability domains of scientifically validated assays and methods that are currently being used so that chemicals can be tested appropriately in these assays to produce valid predictions. In addition, accurate definition of the applicability domains will facilitate modification and improvement of current and new assays to expand these domains, resulting in better coverage of the chemical space for prediction of sensitization potential. The purpose of this Workshop is to cover current knowledge on the applicability domains of these methods, to understand their limitations and the opportunities they offer. The session will open with a brief introduction by the session Chair followed by five presentations aimed at defining the current state of the applicability domains for in vitro approaches as well as recent progress to expand these domains. The first speaker will present the current status of an international collaboration charged with establishing international test guidelines for nonanimal testing strategies that would serve as full replacements to the animal tests for skin sensitization. The approach includes using the current nonanimal methods within their applicability domains to model skin sensitization. The second speaker will define the applicability domains and limitation for the individual OECD Test Guidelines 442c, 442d, and 442e, which address chemical peptide reactivity, keratinocyte activation, and dendritic cell activation, respectively. A complete understanding of the influence of chemistry on these factors is critical for accurate predictions and improvement of current animal models and expand the applicability of chemical compounds to be tested without the use of animals. The third speaker will focus on in vivo assessment of peptide reactivity, presenting a characterization of the currently validated Direct Peptide Reactivity Assay (DPRA) approach while providing insight into the novel Peroxidase Peptide Reactivity Assay (PPRA), which holds promise to expand the applicability domains of nonanimal methods to include pre- and pro-haptens requiring metabolism. The fourth speaker will provide initial data for a novel dendritic cell activation assay, the THP-1 Activation Assay, which is being developed to expand the applicability domain of the current in vitro dendritic cell activation assays to include drugs that exhibit limited or no genotoxicity. The final speaker will introduce the state of the art for novel in vitro approaches based on transcriptional profiling and machine learning to predict sensitization potential. These assays are showing promise for addressing issues of difficult-to-test substances including hydrophobicity, metabolism, solubility, and formulation, and the GARDskin assay will be used to illustrate the progress. Overall, this Workshop aims to better define the applicability domains for nonanimal sensitization testing and identify progress and opportunities for expanding these domains. This Workshop represents an international collaboration between the Immunotoxicology Specialty Section of SOT and the Immunotoxicology and Chemical Allergy Specialty Section of EUROTOX in an effort to communicate and improve the science of alternative approaches for assessing potential for skin sensitization.

Over the past 20 years or more, investigators have been developing non-animal test methods for use in assessing the skin sensitization potential of chemicals. In parallel with this effort, the key biological events of skin sensitization have been well-characterized in an Adverse Outcome Pathway (AOP) for non-animal testing strategies that would serve as full replacements to the test for skin sensitization, a regulatory requirement across many jurisdictions and chemical sectors. A detailed evaluation framework was applied to DAs for hazard identification and potency categorization, including considerations on applicability domain, confidence, uncertainty, and curation of reference data. Based on the progress made in this area, there have been multiple developments in the U.S. regulatory sector, with a draft science policy from US EPA in 2018 to accept two non-animal DAs as replacements for the mouse test, and guidance from the US FDA in 2020 to consider batteries of in vitro and in silico tests in nonclinical safety evaluation of the immunotoxic potential of drugs and biologics. Work is ongoing to target unresolved challenges for risk assessment, such as potency estimation and assessing DA performance on mixtures.

The purpose of this presentation is to present and discuss the applicability domains for these assays and possible limitations of their predictive capacities. The idea is to integrate this simple method during drug development to identify the potential of drugs to induce hypersensitivity reactions in the pre-clinical phase. While without any doubt, additional efforts and extensive resources are necessary to improve preclinical testing methodologies, including optimization of the experimental design and interpretation of data, the possibility of using currently in vitro methods for the hazard identification of the allergenic potential of chemicals for drugs will be presented and discussed.
has required technical adaptations, including use of alternative solvents and extractions vehicles, and optimization of test concentrations for substances of unknown molecular weights. Dose-response analysis of the transcriptomic fingerprints may also provide quantitative estimates of sensitizing potency. In summary, this talk will demonstrate the potential for "in vitro" assays based on toxicogenomics, to fill data gaps where currently validated assays have shown technical limitations, thus contributing to an expanded applicability domain of non-animal-based testing strategies for skin sensitization hazard and potency assessment.

The US Consumer Product Safety Commission (CPSC) received a petition in 2015 from a coalition of organizations and individuals representing physicians, patients, fire fighters, and consumers. The petition requested CPSC to ban non-polymeric, additive Organohalogen Flame Retardants (OFRs) as a class in four categories of consumer products. Due to the breadth of products and chemicals involved in the petition, CPSC sponsored a study through the National Academy of Sciences (NAS), and the report was released in 2019. NAS developed a class approach to hazard assessment and discussed the challenges with its application in the regulatory setting. This aim of this Workshop is to discuss the potential of approaches to evaluating OFRs. The Workshop will address issues such as (1) why OFRs are important for fire safety; (2) what level of data is needed to assess chemical classes; (3) what activities are being applied by other regulatory agencies for OFRs; and (4) whether new approach methodologies (NAMs) and read-across methods are ready for this regulatory application. This session will begin with an overall introduction by the Co-Chair, Linda Birnbaum, a Scientist Emeritus and former Director of NIEHS and NTP. The first speaker, who is leading the CPSC Toxicology and Risk Assessment group, will cover the CPSC perspective on the OFR project, including the petition, the 2019 NAS report, and past and current CPSC work related to OFRs. The second speaker, from the US EPA, will describe a broader overview of the global regulatory landscape. The EU issued bans on the production and use of polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD), starting in 2002. Recent EU regulations related to OFRs include Registration, Evaluation, Authorization and Restriction of Chemicals (REACH), the restriction of hazardous substances (RoHS), the European Electrical and Electronic Equipment (WEEE) directives. Until now only a few of the commercially available BFRs, ca. 75 in total, have been banned, while several others have also been identified in dust from homes and offices, and in plastic consumer products, such as for example 2,4,6-triis(2,4,6-tibromophenoxy)-1,3,5-triazine (TTBP-TAZ). The very high production and high levels of chlorinated paraffins (CPs) have so far not led to restrictions in use or import, although short-chain CPs have been labelled as persistent organic pollutant under the Stockholm Convention. Some of the chlorinated phosphorus FRs (PFs) are in discussion or in preparation for further legislation by the European Chemicals Agency (ECHA). The current Community Rolling Action Plan (CoRAP) of the European Chemicals Agency (ECHA), which prioritizes substances for risk evaluation, contains some OFRs that overlap with the CPSC activities. The third speaker, a toxicologist from industry, will discuss why OFRs are used in consumer products; how OFRs are incorporated into different materials; the mode of action by which OFRs retard fire; and how to balance the fire risk and health risk associated with OFRs. The fourth presentation, given by two speakers who are leading the US EPA Toxic Substances Control Act (TSCA) effort on prioritization and risk evaluation of chemicals (REACH), the Restriction of Hazardous Substances (RoHS), and the European Electrical and Electronic Equipment (WEEE) directives. Until now only a few of the commercially available BFRs, ca. 75 in total, have been banned, while several others have also been identified in dust from homes and offices, and in plastic consumer products, such as for example 2,4,6-triis(2,4,6-tibromophenoxy)-1,3,5-triazine (TTBP-TAZ). The very high production and high levels of chlorinated paraffins (CPs) have so far not led to restrictions in use or import, although short-chain CPs have been labelled as persistent organic pollutant under the Stockholm Convention. Some of the chlorinated phosphorus FRs (PFs) are in discussion or in preparation for further legislation by the European Chemicals Agency (ECHA). The current Community Rolling Action Plan (CoRAP) of the ECHA envisions possible restrictions on a series of chlorinated PFs, tris(1,3-dichloro-2-propyl)phosphate (TDCIPP), tris(2-chloro-1-(chloromethyl)ethy) phosphate (TDCP), and triphenylphosphine (TPhP). BFRs currently under CoRAP include N,N'-ethylene bis(3,4,5,6-tetrabromo-phthalimide), 2,2-dimethylprop-1-ol, tribromo derivative, 1,1’-(isopropylidene) bis[3,5-dibromo-4-(2,3-dibromopropoxy)benzene], N,N’-ethylene bis(3,4,5,6-tetrabromophthalimide), and bis[2-(ethylhexyl)tribromophthalate]. Also on the CoRAP list is antimony trioxide, which is a synergist often used together with BFRs. Antimony trioxide is a class 2 carcinogen and planned to be restricted by the EU in 2019. The EU carried out a risk assessment of antimony trioxide-free BFRs such as poly(pentabromobenzylacrylate), brominated polystyrene and brominated epoxy polymer F2100, all polymers with molecular weights between 20,000 and 600,000. Although the CoRAP program seems to be more progressive than earlier EU approaches to chemical risk assessment, it is unlikely that all OFR and organohalogen FRs (OFRs) will be banned. Alternatives for OFRs are available. However, the lack of pressure from authorities leaves decisions to produce safer alternatives to the industry. Unfortunately, short-term profit from the cheaper OFR production often prevails.

Overview of Organohalogen Flame Retardants (OFRs): Scientific Challenges in Risk Assessment and Potential Regulation


Consumer product-related residential fires are responsible for a large number of deaths and injuries each year in the United States. While flammability standards are effective in mitigating the fire hazard, additive flame retardants are often the most cost-effective way to meet the standards. FR chemicals have been used in multiple products, including home furnishings, wearing apparel, building insulation, and electronics. FRs have been associated with a number of chronic health effects, including cancer and neurotoxicity. Organohalogen flame retardants (OFRs) are a broad class of brominated and chlorinated compounds added to products to reduce flammability. As a result of widespread use, human exposure to OFRs is ubiquitous. In addition, some OFRs are persistent and bioaccumulative. In 2015, a consortium of petitioners asked CPSC to ban the use of additive, non-polymeric OFRs as a class in children’s products, upholstered furniture, mattresses, and electronics enclosures. The petitioners proposed that OFRs had similar biological effects, and that data gaps could be filled through read-across methods. The Commission granted the petition in 2017. As a first step, CPSC sponsored a NAS study on a Class Approach to Hazard Assessment of Organohalogen Flame Retardants, which was published in 2019. NAS concluded that OFRs could not be treated as a single class; rather, they identified 14 subclasses comprising 161 OFRs. NAS outlined a process for conducting hazard identification, using methods including new alternative methods (NAMs) and read-across to fill in data gaps. They identified gap chemicals, dependent on the degree to which the molecules in the subclass are data-rich and the health effects are concordant. Finally, NAS discussed a number of scientific and policy challenges, including the application of read-across methods and NAMs to data-poor chemicals in a regulatory context. This presentation will discuss the scientific and policy challenges associated with a class approach to assessing the potential health risks of multiple classes of chemicals, as well as potential regulation. These comments are those of the CPSC staff, and have not been reviewed or approved by, and may not necessarily reflect the views of, the Commission.

Organohalogen Flame Retardants: The European Situation and Legislation


Recently, several frameworks and directives on the production and use of chemicals have been developed in the European Union (EU), including the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH), the restriction of Hazardous Substances (RoHS), the European Electrical and Electronic Equipment (WEEE) directives. Until now only a few of the commercially available BFRs, ca. 75 in total, have been banned, while several others have also been identified in dust from homes and offices, and in plastic consumer products, such as for example 2,4,6-triis(2,4,6-tibromophenoxy)-1,3,5-triazine (TTBP-TAZ). The very high production and high levels of chlorinated paraffins (CPs) have so far not led to restrictions in use or import, although short-chain CPs have been labelled as persistent organic pollutant under the Stockholm Convention. Some of the chlorinated phosphorus FRs (PFs) are in discussion or in preparation for further legislation by the European Chemicals Agency (ECHA). The current Community Rolling Action Plan (CoRAP) of the ECHA envisions possible restrictions on a series of chlorinated PFs, tris(1,3-dichloro-2-propyl)phosphate (TDCIPP), tris(2-chloro-1-(chloromethyl)ethy) phosphate (TDCP), and triphenylphosphine (TPhP). BFRs currently under CoRAP include N,N'-ethylene bis(3,4,5,6-tetrabromo-phthalimide), 2,2-dimethylprop-1-ol, tribromo derivative, 1,1’-(isopropylidene) bis[3,5-dibromo-4-(2,3-dibromopropoxy)benzene], N,N’-ethylene bis(3,4,5,6-tetrabromophthalimide), and bis[2-(ethylhexyl)tribromophthalate]. Also on the CoRAP list is antimony trioxide, which is a synergist often used together with BFRs. Antimony trioxide is a class 2 carcinogen and planned to be restricted by the EU in 2019. The EU carried out a risk assessment of antimony trioxide-free BFRs such as poly(pentabromobenzylacrylate), brominated polystyrene and brominated epoxy polymer F2100, all polymers with molecular weights between 20,000 and 600,000. Although the CoRAP program seems to be more progressive than earlier EU approaches to chemical risk assessment, it is unlikely that all OFR and organohalogen FRs (OFRs) will be banned. Alternatives for OFRs are available. However, the lack of pressure from authorities leaves decisions to produce safer alternatives to the industry. Unfortunately, short-term profit from the cheaper OFR production often prevails.

Application and Role of OFRs: Understanding Fire Safety and Risk


Fire causes thousands of deaths and tens of thousands of injuries every year worldwide. Most deaths occur in homes and are started by small open flames. While the fuel loads in our domestic environments have increased, the frequencies of fires have decreased in both severity and mortality rates. Several advancements over the past decades have led to the decreases in residential fire-related deaths. This has included the increased use of fire notification and fire protection devices, implementation of robust fire safety standards, and utilization of chemicals to retard fire growth. Flame retardant chemistries play a critical key role in helping products meet fire safety and in reducing the impact of fires on people, property and the environment. These chemistries are incorporated into different materials to reduce the risk of fire either by providing increased resistance to ignition or acting to slow down combustion and thereby delay the spread of flames. In recent years there
has been concern regarding the need to use flame retardants chemicals and their potential health risks thus an improved understanding of flame retardant characteristics, their mode of action and application in products is needed.

1292 Organohalogen (OFR) and Other Flame Retardants: US EPA Toxic Substances Control Act (TSCA) Review Efforts

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Several individual OFRs that are covered by the CPSC under the Federal Hazardous Substances Act jurisdiction are also included as EPA high-priority chemical substances for risk evaluation under TSCA, as amended by the Frank R. Lautenberg Chemical Safety for the 21st Century Act. EPA currently has regulatory activities underway for certain persistent bioaccumulative toxic chemicals (PBTS), including the flame retardants Decabromodiphenyl ether (DecaBDE) and Phenol isopropylated phosphate (PIP 3:1) under TSCA section 6(h). There has been ongoing assessment of structurally similar flame retardants like the Cyclic Aliphatic Bromide Cluster (HBBCD Cluster). HBBCD is included in the first 10 risk evaluations under TSCA and has been peer reviewed. EPA has prioritized for risk evaluation several flame retardants, 4,4’-(1-Methylethylene)bis[2, 6-dibromophenol] (TBBPA), Tris(2-chloroethyl) Phosphate (TCEP), from the TSCA Work Plan as part of the next 20 draft scopes published for risk evaluations. These scopes identify existing reasonably available information relevant for risk evaluation through a systematic review process. Examination of evidence maps for different disciplines will be utilized in a gap analysis to identify critical data needs. EPA will consider its TSCA authorities to obtain appropriate data to fill these critical data needs. In addition, EPA will be exploring tiered testing requirements as outlined under TSCA when examining any data a product might be indicative of vertebrate testing needs. These data needs will also consider appropriate new approach methodologies (NAMs) tests and procedures in filling data needs for risk evaluation.

1293 Human Exposure to Organohalogen Flame Retardants: Sources, Pathways, and Health Concerns

H. Stapleton. Duke University, Durham, NC.

Organohalogen flame retardants (OFRs) are chemicals intentionally applied to various types of textiles, polymers and resins to reduce their flammability, often as a requirement to meet specific flammability standards. Over the last few decades it has become clear that several classes of OFRs are migrating out of treated consumer products and into the indoor and outdoor environment, leading to chronic exposure to both people and wildlife. In the early 2000s, one class of OFRs, polybrominated diphenyl ethers (PBDEs), were phased out due to mounting evidence suggesting that they were bioaccumulative, persistent and toxic. As a consequence, new types of OFRs have now been introduced to the market, and as certain business sectors expand (e.g. electronics and housing), the use of OFRs has also increased. The use of chlorinated organophosphate flame retardants such as Tris (1,3-dichloroisopropyl) phosphate (TDCIPP), which is considered a probable carcinogen, has also following the phase-out of PBDEs. One study found that TDCIPP was the most common OFR applied to baby products (e.g. car seats) and a later study found that TDCIPP exposure was highest in infants compared to older children and adults. Decabromodiphenyl ethane (DBDPE) and 2,4,6-tris(2,4,6-tribromophenoxy)-1,3,5-triazine (TBTBP-TAZ) have both been commonly used in electronic items, such as TV enclosures. Research now suggests that these more recently introduced OFRs are frequently detected in indoor air and dust samples, leading to chronic exposure to the population, particularly in children. Studies have found that levels in indoor air and dust are positively and significantly correlated with biomarkers of exposure in human tissues, suggesting that time spent indoors is a large contributor to overall exposure. This has spurred more research to understand what products are contributing to this exposure, and to evaluate potential health risks. Newer research investigating toxicity and health effects, both in animal studies and human epidemiological studies, raise concerns particularly about thyroid disease, including thyroid cancer, immunotoxic effects (allergies and asthma) and neurodevelopmental impacts following exposure. This presentation will summarize current research on exposure pathways in the general population, possible sources of exposure, and data on potential toxicity.

1294 Controlling the Message: Safely Navigating the Development of Novel Oligonucleotide Therapeutics

J. E. Sutherland. Alnylam Pharmaceuticals Inc., Cambridge, MA.

Oligonucleotide-based therapeutics that utilize nucleic acids to treat a variety of localized or systemic diseases are very novel and relatively unknown to the public. This unique class of agents includes antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs), aptamers, microRNA (miRNA) inhibitors and mimics, and modified messenger RNA (mRNA), which usually require special systems for delivery to patients and have specific safety considerations. In the last 20 years, several ASOs have been approved for the treatment of serious diseases, including fomivirsen for cytomegalovirus retinitis; mipomersen for homozygous familial hypercholesterolemia; nusinersen for spinal muscular atrophy; and inotersen for polyneuropathy caused by hereditary transthyretin-mediated hATTR amyloidosis. In 2018, patisiran, the first RNAi therapeutics, was approved for the treatment of polyneuropathy of hATTR amyloidosis, Givosiran, another RNAi therapeutic, was approved in 2019 for the treatment of acute hepatic porphyria. A robust nonclinical and clinical pipeline features other ASOs, siRNAs, and modified mRNA designs to treat systemic and local diseases in a variety of organ systems. This Symposium will provide current updates on the status of the ASO, siRNA, and modified mRNA platforms, their respective mechanisms of action, their chemistry, and some of the challenges each modality must overcome to enable effective systemic and localized delivery to target tissues, with an acceptable benefit-to-risk profile. Pharmaceutical development of these agents constitutes a prime example of bridging cutting-edge research with the established regulatory requirements allowing for clinical testing. Effective nonclinical safety assessment and the regulatory requirements for evaluating the risks and benefits of these molecules, including appropriate species selection and determination of safety margins, are of paramount importance in bringing these novel therapeutics to patients. Following this session, attendees will have a better understanding of the current thinking surrounding nonclinical safety evaluation and development strategies for this important emerging class of oligonucleotide-based therapeutics.

1295 Using a Toxicology Database to Better Understand the Species Relevance and Safety Attributes of 2’-MOE ASOs

S. P. Henry. Ionis Pharmaceuticals, Carlsbad, CA.

The development of antisense oligonucleotides (ASOs) has been a journey of optimizing chemistry, sequence, and screening methods to obtain the best drug candidates. Given the platform nature of this class of drugs, there is an opportunity to collect data across multiple programs to guide the nonclinical safety assessment in unprecedented ways. To exploit this experience, a database has been established that is comprised of individual animal and time point data from toxicology studies that represent >2000 monkeys treated with 2’-MOE ASOs and >600 monkeys treated with GalNAc-conjugated 2’-MOE ASOs that are derived from >40 compounds. This collective experience is particularly useful as the diversity of antisense applications expands with different routes of administration, chemical modifications, and tissue-targeting strategies. The ability to draw from data that represents multiple closely related compounds facilitates the identification of the most reliable biomarkers for common target organ effects, interpretation of the relevance of toxicology findings to humans and definition of safety margins. These data have also been very informative in identifying the most appropriate species to use in choosing lead compounds and toxicology studies, leading to reduced number of studies with efficient study design. A large database is particularly important in the case of low incidence findings in toxicology studies. An example is the greater understanding of the incidence, dose-response, reversibility, and mechanism of thrombocytopenia in monkeys. Efforts are underway to understand the genomic or immunologic factors that influence individual animal sensitivity. Thus, this database helps inform the appropriate safety monitoring in clinical trials within the context of the disease background. There is also an opportunity to use these data to tailor the nonclinical safety program to minimize animal use while efficiently supporting the investigation of rare diseases.

1296 Toxicological Assessment of RNAi Therapeutics

J. A. Dwybowski. Alnylam Pharmaceuticals Inc., Cambridge, MA.

Short interfering RNA (siRNA) molecules are a new class of human therapeutics that selectively target the endogenous RNA interference (RNAi) mechanism controlling the translation of RNA to protein. Through this mechanism,
RNAi therapeutics can be designed to specifically target mRNA and silence the production of disease-associated proteins and translation associated with viral replication. RNAi therapeutics are synthetic double-stranded RNA molecules that have been chemically modified (e.g. alterations to sugar moiety, phosphate backbone, nucleobase, termini and conjugation groups) to enhance stability and specificity. Intracellular drug delivery to specific target tissues or organs relies on the use of encapsulating formulations (e.g. lipid nanoparticles) or the conjugation to ligands (e.g. N-acetylgalactosamine (GalNAc)) for receptor-mediated endocytosis. The unique physical chemical and pharmacological properties of siRNA molecules present challenges to the design of a nonclinical safety testing strategy that requires the leveraging of guidance for both large and small molecule therapeutics.

This presentation will provide an overview with examples of the factors that need to be taken into consideration when designing a nonclinical toxicology testing strategy including route of administration, impact of ADME properties, species selection, and appropriate use of surrogate compounds.

An overview summarizing the toxicological properties of RNAi therapeutics commonly observed in nonclinical species will be provided along with a discussion of the relevance of these findings to patient safety. Additionally, approaches to addressing safety assessments for special populations (pediatric and renally impaired) will be presented.

1297 Delivering on the Promise of mRNA Therapeutics
J. J. Senn, Moderna Inc., Cambridge, MA. Sponsor: J. Sutherland

mRNA-based therapies have enormous potential in almost every therapeutic area. However, mRNA as a drug is complicated by the biological lability of mRNA and differing mRNA concentrations in the body intracellularly. To conquer this, we have developed technology for the delivery of nucleic acids. Specifically, the use of lipid nanoparticles (LNPs) offers an efficient way to stabilize mRNA and to deliver it intracellularly. These lipid nanoparticles are composed of 4 main components, an ionizable lipid such as MC3, phospholipid, cholesterol and a pegylated lipid for stability. Although effective, MC3-based systems appeared to be limited by immune-mediated reactions that drive both limitations in repeat dosing and safety (namely complement activation and recognition by immunoglobulins). Interestingly, these types of responses have been observed with other therapeutic approaches using liposomes and/or LNPs, particularly pegylated particles. Herein we demonstrate that these Lgs were capable of interacting with the particles, activating complement and led to the redistribution of these particles to the reticuloendothelial system (RES) resulting in unintended immune activation and a lack of continued protein production with repeated dosing. We undertook evaluation of proprietary pegylated lipids that were specifically designed to avoid recognition by the immune system in conjunction with the development of novel amino lipids that were more readily biodegradable and potent. The data presented here suggests that the PEG component was a key player in both the ability to repeat dose safely and effectively. Modification of the PEG component of our proprietary LNPs has allowed reduced or eliminated the accelerated blood clearance observed with historical particles, allowed the ability to IV bolus dose and significantly increased the therapeutic index of these products.

1298 Oligonucleotide Therapeutics: Current Regulatory Considerations and What We Have Learned from the Submission Data to US FDA
X. Chi, US FDA/CDER, Silver Spring, MD. Sponsor: J. Sutherland

This presentation will provide an overview of oligonucleotide (ONT) regulatory landscape in terms of current guidance applicability and some special considerations based on up-to-date FDA experience with various classes of ONT submissions. ONT submissions to the Center for Drugs and Evaluation (CDER) at the Food and Drug Administration has increased substantially over the years. Over 300 ONT Investigative New Drug (IND) submissions and 17 New Drug Applications (NDAs) have been received by the Center in the past 27 years and the submissions span a wide variety of ONT classes, route of administrations (ROAs) and therapeutic areas. ONTs lie at the interface between small molecules and biology in terms of physicochemical and toxicological properties. The nonclinical support required for clinical development tends to be determined case-by-case, and a hybrid of ICH M3(R2) and ICH S6 is generally applied. The requirement is also adapting to evolving knowledge and accumulating data in this new therapeutic modality. To help further in refining the regulatory requirements, the ONT Toxicology Coordinating Committee –ONT Subcommittee is developing an ONT Nonclinical Database on data submitted to the Center. Some findings in the database will be discussed, including the selection of toxicity species of major ONT classes, as well as the incidence of some dose-limiting toxicities in selected toxicology species. Once clear patterns are identified, these data can be used to guide the interpretation of future regulatory data and contribute to the development of position papers or guidance documents.

1299 From Conception to Cane: Unique Life-Stage Considerations for Reproductive Toxicity
M. E. Kossack, Brown University, Providence, RI.

The impact of toxicant exposures on the reproductive system is highly dependent on life stage. During the fetal period, germ cells migrate to the urogenital ridge, the indifferent gonad differentiates into the testis or ovary, and meiosis is initiated in the ovary. In rodent models, reproductive system development continues during the early postnatal period, making the developing gonad sensitive to toxicant exposures during the pregnancy and lactation periods. Once the gonad is fully developed, women and other female mammals have a finite number of oocyte-containing follicles. Throughout the fertile life span, these follicles grow and become capable of releasing oocytes for fertilization. Since the follicular reserve is not renewable, the inherent endowment is gradually depleted with age. Exposure to some toxicants can accelerate reproductive aging, shorten the reproductive life span, or lead to adverse reproductive outcomes in the children of aging parents. As the reproductive life cycle is started again with the next generation, epigenetic alteration incurred throughout a lifetime of environmental exposure may be passed on to subsequent generations. Each period of the reproductive life span is vulnerable to the perturbations from environmental chemicals resulting in reproductive toxicity. This Scientific Session will address the effects of life stage-specific reproductive toxicity. Beginning with pregnancy, through prenatal development, aging, and transgenerational effects, this session will explore how chemical exposure affects reproductive potential in both males and females. Further, the speakers will use case examples in human as well as mammalian and fish models to investigate the life stage-specific risk of exposure to environmental chemicals.

1300 Endocrine-Disturbing Chemicals in Pregnant Women and Potential Modifying Factors
R. S. Strakovsky, Michigan State University, East Lansing, MI.

Pregnancy is a sensitive window for both maternal and child health. In animal models and human epidemiological studies, maternal exposure to endocrine disrupting chemicals (EDCs) has been shown to be detrimental for both pregnancy outcomes and fetal development. This is concerning because virtually all pregnant women are exposed to certain classes of EDCs, including phthalates and parabens, which are found in food contact materials and personal care products. This presentation will focus on studies that assess associations of chemicals with maternal endocrine dysregulations in a cohort of pregnant women, and will provide examples of how these disruptions relate to fetal growth and development. An additional important emphasis will be on potential modifiers of these relationships. For example, similar to findings in animal models and other epidemiological studies, our data suggest that relationships between EDCs and birth outcomes differ by the sex of the fetus. We also observe that these relationships may differ by numerous other maternal health and lifestyle factors, including maternal pre-pregnancy weight and diet. There are several reasons to consider these modifying or modifying factors when evaluating associations of EDCs with pregnancy-related outcomes. First, these complex relationships in pregnant women may help to explain certain incongruencies between observations in human populations and animal models, especially those related to impacts of EDCs on specific hormonal pathways. Second, these types of studies will allow us to provide public health messages that focus on modifiable lifestyle factors capable of mitigating the negative impacts of EDC exposures in pregnancy.

1301 Disruption of Retinoic Acid Signaling: A Mechanism of Phthalate Toxicity in the Seminiferous Cord
D. Spade, Brown University, Providence, RI.

Susceptibility of the fetal testis to phthalic acid esters (phthalate) toxicity has been recognized for several decades. However, aspects of phthalate toxicity mechanisms, including molecular initiating events, have not been described. Phthalates with medium-length side chains disrupt androgen biosynthesis and reduce fertility, especially in the seminiferous cord development in animal models. The strength of the anti-androgenic effect is greater in rats than in mice and does not appear to be directly responsible for the effects of phthalates on the seminiferous cord. In addition to being anti-androgenic, phthalates are peroxisome pro-
Environmental chemical exposures can elicit heritable health effects, primarily when they occur during sensitive windows of embryonic development. Environmentally induced phenotypic traits have been found to be inherited by subsequent generations even after the exposure occurred several generations ago. These effects that develop in the absence of exposure but result from ancestral exposure are called transgenerational health effects.

Transgenerational health effects in humans are not clearly understood; however, studies in non-human experimental animal models suggest that chemicals can leave exposure-specific epigenetic marks on germline cells that are transmitted to subsequent generations resulting in adverse health outcomes. Multigenerational health effects, which are caused by direct contact with the chemical, seem to be different from transgenerational health effects. We are studying mechanisms underlying the developmental origins of adult and transgenerational adverse health outcomes using Japanese medaka (Oryzias latipes) as an animal model. Embryonic BPA (100 μg/L), EE2 (0.05 μg/L), and atrazine (5 μg/L) exposure did not induce phenotypic abnormalities in F0 males in adulthood, whereas a significant reduction in fertility was observed by six months of age, zebrafish developmentally exposed to 0.5 μM CBD showed decreased fecundity and when aged (30 months old), this treatment significantly reduced zebrafish sperm concentrations. The developmental exposure also resulted in aged fish with significantly increased survival (~20%), reduced size (weight and length) and reduced expression of several genes including tnfa, il-1b, il-6, and PPARγ; supporting the need to consider the developmental origins of health and disease of CBD. Furthermore, availability of mutant fish lines and the ability to quickly screen pharmacological inhibitors facilitate study of molecular mechanisms of CBD efficacy and toxicity. For example, using cannabinoid receptor-null zebrafish, we found that CBD-induced malformations (pericardial and yolk sac edemas) and mortality were significantly reduced in the cnr1-/- and cnr2-/- larvae compared to cnr+/-, Transscriptomic profiling in cnr+/-/embryos developmentally exposed to 0.5 μM CBD revealed that a significant portion of differentially expressed genes were targets of PPARα, a predicted upstream regulator. In both cnr- positive and cnr-null embryos co-exposed to the PPARα inhibitor GW9662 and CBD, there was increased toxicity compared to exposure with CBD alone suggesting that PPARα, cnr1, and cnr2 all play roles in the developmental toxicity of CBD.

Zebrafish as a research organism provide a relatively high-throughput model by which both the efficacy and potential toxicity of CBD can be assessed. For example, using zebrafish that carry a mutation in the voltage-gated sodium channel Nav1.1 (scn1α) to replicate the human Dravet syndrome, we found significant anti-seizure activity with 0.6 μM CBD after 24 h larval exposure. Despite the significant therapeutic potential of CBD, toxicity assessments particularly for developmental or chronic exposures are lacking. To assess both the acute and developmental consequences of CBD, zebrafish were exposed through the larval stage to 0.02 to 4 μM CBD. In larval zebrafish, important differences in cannabinoid bioavailability were identified, specifically the bio-accumulation of CBD was significantly higher than δ-9-tetrahydrocannabinol. By six months of age, zebrafish developmentally exposed to 0.5 μM CBD showed decreased fecundity and when aged (30 months old), this treatment significantly reduced zebrafish sperm concentrations. The developmental CBD exposure also resulted in aged fish with significantly increased survival (~20%), reduced size (weight and length) and reduced expression of several genes including tnfa, il-1b, il-6, and PPARα; supporting the need to consider the developmental origins of health and disease of CBD. Furthermore, availability of mutant fish lines and the ability to quickly screen pharmacological inhibitors facilitate study of molecular mechanisms of CBD efficacy and toxicity. For example, using cannabinoid receptor-null zebrafish, we found that CBD-induced malformations (pericardial and yolk sac edemas) and mortality were significantly reduced in the cnr1-/- and cnr2-/- larvae compared to cnr+/-, Transscriptomic profiling in cnr+/-/embryos developmentally exposed to 0.5 μM CBD revealed that a significant portion of differentially expressed genes were targets of PPARα, a predicted upstream regulator. In both cnr- positive and cnr-null embryos co-exposed to the PPARα inhibitor GW9662 and CBD, there was increased toxicity compared to exposure with CBD alone suggesting that PPARα, cnr1, and cnr2 all play roles in the developmental toxicity of CBD.

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The Global Cannabidiol Market was 7.1 Billion in 2019, Predicted to be 9.3 Billion in 2020. The Market is expected is expected to grow at a compound annual growth rate (CAGR) of 22.2% from through 2025. CBD products are associated the multiple therapeutic claims ranging from chronic pain to arthritis, anxiety, sleep etc. The widespread use of CBD oils increases associated efficacy and safety risks. As such exploratory research needs to inform the risks associated with CBD as well as multiple cannabinoids and terpenes present in full spectrum extracts. We will explore the relative contribution of the entourage effect associated with multiple facets of full spectrum cannabidiol oil that can impact the margin of safety for a particular complex cannabidiol product in the marketplace.
Cannabidiol (CBD) is a plant-derived cannabinoid similar in structure to the psychoactive cannabinoid, 79-tetrahydrocannabinol (THC), although CBD does not produce a high. Despite their differences in psychotropic effects, both THC and CBD are immune suppressive. We utilized the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis to determine effects and mechanisms by which CBD suppresses immune function in vivo. EAE disease was initiated on day 0 followed by 5 days of dosing with CBD by oral gavage. CBD significantly reduced disease severity at day 18. T cell responses in the spleen, lymph nodes, spinal cord and cerebellum were assessed at day 3, 10 and 18 following disease initiation. IFN-gamma production was significantly inhibited by CBD in the spleen at day 10, which preceded suppression of inflammation in the spinal cord and cerebellum at day 18. In vitro studies revealed that CBD suppressed IFN-gamma production in response to several different stimuli. Moreover, CBD treatment at the time of cellular activation resulted in robust suppression of IFN-gamma. Together these data demonstrate that CBD suppresses IFN-gamma suggesting that it might be effective for autoimmune diseases, but also requires that caution be exercised in its broad use because of the potential to suppress immune function.

In June 2018, the FDA approved the first drug comprised of an active ingredient of cannabis—a highly purified oral solution of cannabidiol. With rescheduling, it is now available for use in clinical practice with an indication to treat refractory seizures associated with 2 severe childhood onset epilepsy syndromes: Lennox Gastaut Syndrome (LGS) and Dravet Syndrome (DS). This talk will focus on safety issues as they apply to this pharmaceutical grade CBD product, given the high quality and controlled data available for this particular product. First, an introduction to the endocannabinoid system will be presented, which will set the stage for a brief review of CBD’s pharmacology as it is relevant to the treatment of seizures will be presented, including proposed mechanisms of action and metabolism. Based on CBD’s various actions on the cytochrome P450 system, concerns arise for drug-drug interactions. In vivo and in vitro data regarding potential pharmacodynamic and pharmacokinetic drug-drug interactions with CBD will be discussed. Data to support and refute CBD-clobazam interaction will be presented. Interactions with valproate, tacrolimus, and anticoagulants will be discussed. The data available on the effect of food and CBD absorption will be discussed. Clinical trials showed higher incidence of anemia in patients treated with CBD vs. placebo. Approximately 30% of patients taking CBD developed laboratory defined anemia vs. 13% in placebo. A 10% increase serum creatinine was seen in healthy adults, LGS and DS patients. When cannabidiol (75, 150, or 250 mg/kg/day) was orally administered to rats throughout pregnancy and lactation, decreased growth, delayed sexual maturation, neurobehavioral changes (decreased activity), and adverse effects on male reproductive organ development (small testes in adult offspring) and fertility were observed in the offspring at the mid and high dose. These effects occurred in the absence of maternal toxicity. Recommended safety monitoring in patients taking CBD will be discussed.

This talk will focus on the regulatory concerns related to cannabidiol (CBD) commercialization and build on the information conveyed by preceding speakers related to the science behind CBD as an ingredient with biological properties that impart desirable effects. The ever-changing regulatory landscape surrounding use of cannabis-related ingredients in consumer goods in the US was further complicated in 2019 by the passage of the Farm Bill which has legalized and expanded the cultivation of hemp and the production of hemp-derived CBD across the US. This talk will provide an update on the regulatory oversight of CBD as it relates to its incorporation into a wide variety of consumer products (e.g., homeopathic drug products, foods, dietary supplements, beverages, and cosmetics), as well as uses in CBD products as an ingredient for addition to this wide variety of consumer goods. Both federal and state regulatory bodies are involved in various aspects of CBD product production, distribution, and marketing; this talk will focus on public health and safety concerns related to CBD commercialization. The FDA policy in 2020 is that it is not currently lawful to add CBD to human or animal food or to market CBD products as dietary supplements. Therefore, examples of regulatory actions that have been taken by either the federal government or state governments will be discussed, with these examples taken from the most recent regulatory actions. Examples may include (1) recent US FDA actions to crack down on the use of certain unsupported health claims on consumer products containing CBD; (2) US FDA actions in the area of analytical testing of CBD-containing products and findings that certain products contained “unapproved new drug products”; (3) actions by States to seize/embargo consumer products sold as foods (baked goods, beverages, etc.) because CBD is not a legal food additive that has been proven safe for use; and (4) actions by States whose laws have not kept up with recent Federal law related to hemp production and sale of CBD products and have resulted in actions against truckers moving the products across state lines to confiscation of products in stores.
Regulatory toxicologists apply the interdisciplinary science of toxicology to real-life public health problems within the boundaries of legislative and political frameworks. In this context, testing and assessment methodology is often highly standardized to achieve acceptance of risk assessment methodology for harmonization on an international scale. For research projects in this field, the close interaction with regulators can help to better target regulatory needs, thereby enhancing the practical impact of the project. In this presentation, practical experience with the interaction between academic researchers and regulators are reported from the EU-ToxRisk project. Since the start of the project, EU-ToxRisk has initiated collaborations with regulators from national, European, and international regulatory authorities, formalized in its ‘Regulatory Advisory Board’ (RAB). This cooperation has resulted in an improved mutual understanding of the requirements and pitfalls of NAM-supported risk assessment, from a scientific and regulatory perspective. EU-ToxRisk has found the various interactions with the wider community of regulatory stakeholders crucial, not only in “getting the science right” but equally in “getting the framing and reporting right”. Inter alia, the exchange between researchers and regulators has led to improved case study strategies better focused on regulatory questions, but also to the creation of a NAM-based read-across (RAx) framework and an advisory document for applying NAMs in (regulatory) RAx. Also, templates for better reporting NAMs and their results were developed with the scope of demonstrating their reliability for regulatory purposes. The benefit of this exchange was mutual since participating regulators were not only provided with the chance of discussing cutting-edge science in their field but were also forced to actively reflect their current paradigms and potential role concerning the integration of NAMs into their respective legislative frameworks. This first presentation will depict, horizontally, the most relevant practical examples of how concrete communication interfaces have been established between regulators and researchers, which barriers have been encountered, and which solutions have been proposed to overcome them. Learnings will be dissected from the perspective of a regulatory agency’s representative and RAB’s chairman.

Next-generation risk assessment (NGRA) is an area of intensive research. It assesses the hazard and exposure of compounds purely based on non-animal approaches by using human-relevant in vitro and in silico models. NGRA requires the integration of different expertise and therefore a close interaction within a multi-disciplinary research team. Providing confidence in the testing strategy and fostering its regulatory implementation require extensive evaluation of these new methodologies. Case studies are an excellent tool to assess the performance of in vitro test systems and, overall, the integrated testing strategy, not only to learn more about limitations and remaining uncertainties but also to delineate acceptance criteria together with stakeholders from regulatory agencies. This talk will focus on the experience of the EU-ToxRisk project in conducting case studies. "Running" a case study encompasses an iterative process of testing and evaluation. The design of a case study starts from the definition of the target chemical and the corresponding regulatory hypothesis. Subsequently, a case study team identifies all relevant data gaps and then delineates proper methods to fill them. EU-ToxRisk’s case studies addressed a range of regulatory contexts and endpoints, including liver steatosis, Central Nervous System (CNS) degeneration, lung fibrosis, and developmental toxicity. Following experimental testing, case study teams evaluated, compared, and integrated study results, thereby clearly stipulating what each method contributed. The iterative testing and evaluation process coupled to seeking alignment with all stakeholders led to an increased understanding of the utility of NAM in chemical safety assessment, and thus helped demarcate the applicability domain in NGRA. In this presentation, a group of case studies focused read across of branched carboxylic acids related to liver steatosis and read across of mitochondrial complex inhibitor-mediated CNS degeneration will be outlined including their design process, their implementation and performance in dealing with different regulatory questions and scenarios.
be used to address some of these issues, but they have limitations of their own that need to be addressed prior to use in regulatory decision-making. An important step is to develop mechanisms to bring together government entities to discuss limitations and barriers to acceptance of NAMs, and ideally to collaborate on possible solutions. This presentation will provide relevant ongoing examples of such activities. The EU-ToxRisk consortium has promoted several initiatives aiming to forge international key opinion leaders. Significant examples include the workshops organized in the context of read-across implementation and acceptance. These will be discussed along with related programs, such as APCRA (Accelerating the Pace of Chemical Risk Assessment) and the OECD’s Integrated Approach to Testing and Assessment (IATA) Case Studies Project. APCRA is a government to a government initiative that brings together regulatory agencies from around the world to discuss and initiate collaborative case studies to advance approaches in the development and implementation of NAMs for hazard, exposure, and risk assessment methods for specific decision contexts. Similarly, the IATA Case Studies Project develops case studies to share and explore the use of novel methodologies within specific regulatory contexts. This presentation will give an overview of the mentioned collaborative mechanisms, focusing on their relevance for reaching international harmonization. The discussion will highlight the progress and barriers derived by the proposed activities in establishing global confidence in the use of NAMs. Examples of collaborative international case studies designed to address key issues in applying NAMs to prioritization, screening, and quantitative risk assessment for regulatory decision-making, will be finally described. Disclaimer: The views expressed are those of the author and do not necessarily represent the policies of the US EPA.

2000 Chlorpyrifos induces Cell Proliferation in MCF-7 and MDA-MB-231 Cells, through Cholinergic and Wnt/β-Catenin Signaling Disruption, AChE-R Upregulation, and Oxidative Stress Generation after Single and Repeated Treatment

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Chlorpyrifos (CPF), an organophosphorus (OP) biocide, has been associated with a higher breast cancer risk in women and with breast tumors induction in rats. However, the processes behind are yet to be uncovered. CPF was shown to increase MCF-7 and MDA-MB-231 cells proliferation partially through KIAA1363 overexpression and aryl hydrocarbon receptor activation after acute and long-term treatment, but also through estrogen receptor-alpha activation after 24 h exposure in MCF-7 cells, suggesting other mechanisms should be involved. Otherwise, CPF was reported to induce reactive oxygen species (ROS) generation, acetylcholine (ACh) accumulation, overexpression of acetylcholinesterase-R/S (AChE-R/S) variants and alter the Wnt/β-catenin pathway, both in vitro and in vivo in process different from cancer. These mechanisms were related with cell proliferation. Therefore, we hypothesized that CPF (0.01-100 µM) could mediate the cell proliferation observed after one and fourteen days of treatment in MCF-7 and MDA-MB-231 cells through the common mechanism of action. Our results prove that CPF, following single and repeated treatment, induced ROS generation and lipid peroxidation, ACh accumulation due to AChE inhibition, Wnt/β-catenin up- or downregulation depending on the CPF treatment concentration, and AChE-R and AChE-S overexpression, being only the latter mediated through GSK-3β activity alteration depending on the CPF treatment concentration, and AChE-R and AChE-S overexpression, being only the latter mediated through GSK-3β activity alteration. Wnt/β-catenin signaling disruption. The present data provide novel information of CPF effect on human breast cancer cell lines that could help explain its involvement in breast cancer.


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The histone methyltransferase G9A (EHMT2) gene catalyzes methylation of histone 3 lysine 9 (H3K9) and this gene silencing activity contributes to the tumor promoter-like activity of G9A in several tumor types including alveolar rhabdomyosarcoma (ARMS). Previous studies show the orphan nuclear receptor 4A1 (NR4A1, Nur77) is overexpressed in rhabdomyosarcoma and exhibits pro-oncogenic activity. In this study, we show that knockdown of NR4A1 in ARMS cells decreased expression of G9A mRNA and protein. Moreover, treatment of ARMS cells with several bis-indole - derived NR4A1 ligands (antagonists) including 1,1-bis(3-indolyl)-1-(4-hydroxyphenyl)ethane (CDMB8), 3,5-dimethyl (3-(CH3)3) and 3-bromo-5-methoxy (3-Br-5-OCH3) analogs also decreased G9A expression. Furthermore, NR4A1 antagonists also decreased G9A expression in breast, lung, liver and endometrial cancer cells confirming that G9A is an NR4A1-regulated gene in ARMS and other cancer cell lines. Mechanistic studies showed that the NR4A1/Sp1 complex interacted with the GC-rich - 511 region of the G9A promoter to regulate G9A gene expression. Moreover, knockdown of NR4A1 or treatment with NR4A1 receptor antagonists decreased overall H3K9me2, H3K9me2 associated with the PTEN promoter and PTEN-regulated phospho-Akt. In vivo studies showed that the NR4A1 antagonist (3-Br-5-OCH3) inhibited tumor growth in athymic nude mice bearing Rh30 ARMS cells and confirmed that G9A was an NR4A1 regulated gene that can be targeted by NR4A1 receptor antagonists.

2002 Comprehensive Chemical Evaluation of Aerosol Constituents from JUUL Virginia Tobacco 5.0% Using Non-targeted Analysis: LC-HRMS Analysis of e-Vaporos, Data Acquisition, and Processing

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JUUL Labs Inc, San Francisco, CA. Sponsor: F. Ayala-Fierro

Exposure to the smoke resulting from tobacco combustion is a primary risk factor for many diseases, including cancer. These health risks are related to the amounts of toxicants in the smoke emissions and lifetime exposure. The JUUL brand system (JUL) is an electronic nicotine delivery system that uses a battery and a heating coil to aerosolize an e-liquid formulation to deliver nicotine. A thorough non-targeted analysis was performed to fully characterize the chemical composition of the aerosol for a toxicological assessment to determine the risks associated with using JUUL as an alternative to conventional cigarettes for adult smokers. For JUUL Virginia Tobacco with 5.0% nicotine (VT5), a total 21 compounds were identified in the aerosol collected using intense puffing regime and 19 compounds in the aerosol collected using the non-intense puffing regime. The reaction products accounted for the greatest number of compounds, 66% and 73% in intense and non-intense, respectively. Among ingredients excluding nicotine, water, propylene glycol, benzyl alcohol, and glycerol, were 14% and 16% of the total number of compounds, respectively. Unrationalized compounds made up 0.00096% and 0.003% of aerosol collected mass, respectively. A total of 18 compounds were found to be common in intense and non-intense aerosol, resulting in a total of 22 unique compounds. The total mass of compounds resulting from ingredient reactions only slightly increased with intense puffing conditions, showing that puffing regimen has little impact on aerosol composition.

2003 Multi-omics Analysis to Identify Unique Biomarkers of Hepatocellular Carcinomas in B6C3F1/N Mice

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Hepatocellular carcinomas (HCCs) are a common endpoint in the rodent cancer bioassay and the tumors arising spontaneously or due to chronic chemical exposure are histologically indistinguishable. We hypothesized that unique biomarkers could help to distinguish these histologically distinct tumors. Whole exome sequencing, RNA sequencing and miRNA sequencing were performed on HCCs that arose either spontaneously or due to chemical exposures in B6C3F1/N mice and on age-matched non-tumor livers from control mice. DNA sequencing and mutation analysis identified distinct mutational signatures in bromodichloroacetic acid (BDCA)-induced HCCs and significantly mutated driver genes, including Hras, Sfi1, Ube2c, Cystin. Differential gene expression and alternative splicing (AS) analyses showed that BDCA-induced HCCs clustered differently from those induced by other chemicals, and spontaneous HCCs clustered with other chemical-induced HCCs. The alternative splicing landscape revealed transcript isoforms in 166 genes (10 cancer genes) that were common to all HCC groups, and in 1146 genes (49 cancer genes) were unique to the individual HCC groups. In addition, unique IncRNAs (8, 9, and 41) and miRNAs (7, 12, and 53) were differentially altered across all HCC groups, unique to spontaneous HCCs and each of the chemical-exposed HCC groups, respectively. Functional analysis indicated that IncRNA-miRNA co-expression analysis showed that hub genes are enriched in the pathways in cancer, cell cycle pathway, PI3K-Akt signaling pathway, and carbon metabolism pathway, across all HCC groups. This integrated multi-omics analysis helps us to better understand chemical-induced hepatocarcinogenesis and identify unique biomarkers to differentiate spontaneous HCCs from chemical-exposed HCCs.
Numerous studies have investigated the role of tobacco smoke in breast cancer risk, with many identifying a positive association. For example, a meta-analysis of 26 studies showed a significant increase in breast cancer risk among women who had ever smoked [1]. This finding has been corroborated by several follow-up studies, indicating a consistent association between smoking and breast cancer incidence [2].

However, the specific mechanisms by which smoking increases breast cancer risk are not fully understood. One possibility is that smoking induces DNA damage, leading to the development of mutations in breast tissue. Recent studies have shown that smoking is associated with an increased risk of DNA damage in breast tissue [3]. This increased DNA damage may increase the risk of subsequent mutations that drive breast cancer development.

Furthermore, smoking is associated with changes in the hormonal environment, which may also contribute to breast cancer risk. For example, smoking is associated with increased levels of estrogen, which is a known risk factor for breast cancer [4]. This hormonal effect may contribute to the increased risk of breast cancer observed in smokers.

In conclusion, smoking is a significant risk factor for breast cancer, with evidence suggesting a direct link between smoking and DNA damage in breast tissue. Further research is needed to elucidate the specific mechanisms by which smoking increases breast cancer risk, as this knowledge could inform smoking cessation efforts and other interventions aimed at reducing breast cancer incidence.

References:
exposure to arylamines to that in 26 FFPE bladder tumors from men -out such exposure. Gene expression analysis was performed on NanoString nCounter system using a PanCancer Progression panel comprised of 740 cancer progression-related genes and a custom panel of 69 arylamine- and bladder cancer-related genes. Although fold differences were small, there was evidence of differential expression by exposure status for 17 genes from the Progression panel and 4 genes from the custom panel, with evidence of dose-response in 4 genes. Differentially expressed genes were in pathways related to DNA damage signaling and epithelial-to-mesenchymal transition (EMT). Overall, we found limited evidence of gene expression differences in tumors by exposure status.

2009 A Novel Anthelmintic Drug Suppresses the Growth of Medulloblastoma Tumors by Inhibiting PKA/Gli1 Signaling Axis

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Pediatric brain tumor is one of the most malignant solid tumors in children and have profound impact on the morbidity and mortality in these patients. Statistically, brain tumors are one of the leading cause of cancer-related deaths in patients from 0-19 years of age. Medulloblastoma (MB) is one of the most common pediatric brain tumors occurring in children. Sonic hedgehog (Shh) activated subgroup of MB is considered to be highly aggressive and metastatic in nature. Shh-MB is characterized by mutations in PTCH1, SMO and SUFU along with amplified activation of Gli1, a major transcription factor of this signaling pathway. In the current study, we have evaluated the anti-cancer effects of an anthelmintic drug 'moxidectin'. Several MB cell lines such as Daoy, UW426, UW228, ON576, and PSFK1 were treated with moxidectin in a concentration and time dependent manner. Our results demonstrated that moxidectin treatment resulted in significantly reduced 3D proliferation of MB cells. The IC$_{50}$ of moxidectin in all the MB cell lines ranged between 10-17µM after 24, 48 and 72 hours of treatment. Moreover, moxidectin was able to induce 3-4 fold increase of apoptosis in all the MB cell lines as evaluated by AnnexiniV-FITC/PI assay, and increased cleavage of caspase 3 and PARP.

Western blotting analysis demonstrated that moxidectin treatment significantly reduced canonical activation of Gli1 and its downstream effector molecules such as Pax-6, Oct-4, Sox-2 and Nanog. To our knowledge, this study for the first time focuses on GABA receptor agonist mediated PKA inhibition and ultimately suppression of non-canonical Gli1 activation. Efficacy of moxidectin was evaluated in an in vivo tumor model by subcutaneously and intracranially implanting human Daoy MB cells. Our results demonstrated that 2.5 mg/kg moxidectin by oral administration everyday suppressed the growth of Daoy tumors by 50-55% in both subcutaneous and intracranial tumor models. Conclusively, our results indicate that moxidectin effectively reduces the growth of MB tumors by inhibiting PKA/Gli1 signaling. Most importantly, moxidectin is FDA approved drug and is already in clinical use for the treatment of river blindness in humans with an established safety record, therefore any positive findings from our studies will prompt further clinical investigation into repositioning moxidectin for the treatment of MB patients.

2010 1,2-dichloropropane Induces γ-H2AX Expression in Human Cholangiocytes Only in the Presence of Macrophages


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It was reported that cholangiocarcinoma in workers exposed to 1,2-dichloro-
propane (1,2-DCP) in an offset proof printing factory in Japan. In this cholan-
giocarcinoma, infiltration of inflammatory cells was confirmed under the epi-
theilum cells. In addition, histone family member X (H2AX) phosphorylated on Ser 139 (γ-H2AX), a marker of DNA double strand break, was observed widely in bile duct cells. The present study investigated the effects of 1,2-DCP on the expression of γ-H2AX in human immortalized cholangiocytes MIMNK-1 cells. Monocultures of MIMNK-1 cells and co-cultures of MIMNK-1 cells and THP-1 macrophages were exposed to 1,2-DCP at a concentration of 100 and 500 µM for 24 hours. Expression of γ-H2AX was visualized by immunofluorescence staining. 1,2-DCP had no effect on the relative number of γ-H2AX-expressing mono-cultured MIMNK-1 cells. In contrast, exposure to 1,2-DCP significantly increased the expression of γ-H2AX in MIMNK-1 cells co-cultured with THP-1 macrophages. In addition, the level of the tumor necrosis factor (TNF)-α was significantly increased in the supernatant of MIMNK-1 cells co-cultured with THP-1 macrophages after exposure to 500 µM of 1,2-DCP. The results suggest that macrophages play a critical role in the induction of DNA double strand break in MIMNK-1 cells in the presence of 1,2-DCP.


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Resveratrol (3,5,4'-trihydroxystilbene) is a polyphenolic phytochemical found in fruits, nuts, and vegetables and there is evidence that this compound offers protection from several human diseases including cancer. In cancer cell lines, resveratrol inhibits cell growth, survival, migration/invasion and genes/pathways associated with these anticancer activities. Many of the same anticancer activities reported for resveratrol have previously been observed in this lab- oratory using the nuclear indole derived NR4A1 receptor. NR4A1 binds to and antagonizes of NR4A1-regulated pro-oncogenic pathways. Treatment of A549, H460, H1299 lung cancer cells with 50-125 µM resveratrol for 24, 48, 72 hours inhibited cell growth and IC$_{50}$ values for growth inhibition decreased with time. In addition, resveratrol inhibited the mTOR signaling pathway and other responses in lung cancer cells as previously observed for NR4A1 antagonists in the same cell lines. Therefore, we investigated the interactions of resveratrol with the ligand binding domain of NR4A1 in an assay that measures fluorescent quenching of a tryptophan residue in the NR4A1 ligand binding pocket. Resveratrol bound NR4A1 and the K$_d$ value was 1.4 µM. H460 and H1299 lung cancer cells were transfected with the yeast Gal4-NR4A1 fusion construct and UAS-luciferase which contains tandem Gal4 response elements, and treatment with 125+150 µM resveratrol decreased transactivation. Thus, resveratrol directly bound NR4A1, inhibited NR4A1-dependent transactivation, inhibited cell growth and mTOR signaling, and the role of NR4A1 in mediating the responses induced by resveratrol is currently being investigated.

2012 Cells Exposed Chronically to Hexavalent Chromium Escape Cell Death and Develop Permanent Chromosome Instability

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Hexavalent chromium (Cr(VI)) compounds are known human lung carcino-
gens; but the carcinogenic mechanism is poorly understood. Cr(VI) induces DNA damage which normally leads to apoptotic responses to avoid trans-
formation and carcinogenesis. Evasion of apoptosis is a hallmark of carcino-
genesis, 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 chromate for 6 months (0.0125, 0.025 and 0.05 µg/cm²). Growth parameters, and chro-
mosome instability were measured every 10 days throughout treatment; soft agar growth was measured in the middle and at the end of treatment. Increases in chromosomal alterations were observed beginning at day 10 and increased with time, increases in numerical chromosome instability were ob-
served as foci increases in structural instability. No growth in soft agar occur-
ing during the exposure period. At the end of treatment cells were seeded at colony forming density, surviving colonies for each treatment group were randomly selected as well as an additional set of ‘slow’ emerging colonies, then expanded into cell lines and characterized to determine permanent chromosomal instability. Evasion of normal cells exposed to Cr(VI)-treated clones, that initially emerged after exposure to 0.0125, 0.025 and 0.05 µg/cm² zinc chromate for 6 months, exhibited permanent chromosome insta-
bility in 20, 90 and 70 percent of clones, respectively, long after the removal of treatment. In contrast, all of the slow emerging clones were abnormal. Eighty-five percent of all abnormal clones were highly aneuploid, containing stable translocations but also many unstable numerical and structural changes. In addition, 70% of the abnormal clones were able to grow in soft agar. Future work will identify chromosome specific targets and investigate how they are targeted. These data support a hypothesis that Cr(VI)-treated cells can evade cell death and transform into chromosomally unstable cells that continue to survive and grow. This work was supported by NIEHS grant ES016893 (J.W.P.) and the University of Louisville School of Medicine Basic Grant Program (S.S.W.).

2013 Short Chain Fatty Acids (SCFAs) Act as Selective Estrogen Receptor Downregulators (SERDs) in Mutant ERa MCF-7 Cells

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Resistance to endocrine therapy is commonly observed for estrogen receptor α (ERα) positive breast cancer patients, and this is due in part to mutations in the ligand binding domain of ERα. Two of the most common mutations are ERα-D538G and ERα-Y537S which are constitutively active and are treated with selective ER degraders (SERDs) which are being developed in several
laboratories. Our research has focused on effects of short chain fatty acids as SERDs based on their activity as histone deacetylase (HDAC) inhibitors. MCF-7 cells expressing wild-type and mutant (D538G and Y537S) ER were treated butyrate, propionate, and acetate and this resulted in decreased cell proliferation, decreased ERα expression, and induced Annexin V staining. The HDAC inhibitors Panobinostat, Vorinostat, and Entinostat also decrease wild-type and mutant ERα levels in MCF-7 cells and both SCFAs and HDAC inhibitors enhance histone acetylation. These data, coupled with results of HDAC1 and HDAC6 knockdown suggests that SCFAs and HDAC inhibitors act as SERDs and their mechanism of action involves downstream genes associated with inactivation of HDACs.

The study objective of the present study was to further evaluate potential carcinogenic effects of ETU on the liver, an organ with a high fraction of body weight. The aim of the present work is to investigate the acetylation status of NAT1, as drug metabolizing enzyme that influences cancer cell proliferation and survival, especially in breast cancer. The mechanism for these effects is yet to be determined. Acetylation is an important Post-Translational Modification in the regulation of diverse cellular processes. Histone deacetylases (HDACs) and Sirtuins (SIRT), as protein deacetylases, may have an important role on the NAT1 acetylation status, affecting its catalytic activity. Histone deacetylases (HDAC) and Sirtuins (SIRT), as protein deacetylases, may have an important role on the NAT1 acetylation status, affecting its catalytic activity. Histone deacetylases (HDAC) and Sirtuins (SIRT), as protein deacetylases, may have an important role on the NAT1 acetylation status, affecting its catalytic activity. HDAC6 knockdown suggests that SCFAs and HDAC inhibitors act as SERDs and their mechanism of action involves downstream genes associated with inactivation of HDACs. Enriched canonical pathway analysis found activated oxidative stress-related pathways suggesting oxidative stress may have contributed to the hepatocarcinogenic activity.

**2015 Acetylation of Arylamine N-acetyltransferase 1 in Breast Cancer as a Regulator of Catalytic Activity and Expression**

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N-acetyltransferase 1 (NAT1) is a drug metabolizing enzyme that influences cancer cell proliferation and survival, especially in breast cancer. The mechanism for these effects is yet to be determined. Acetylation is an important Post-Translational Modification in the regulation of diverse cellular processes. Histone deacetylases (HDACs) and Sirtuins (SIRTs), as protein deacetylases, may have an important role on the NAT1 acetylation status, affecting its catalytic capacity and having an impact on the downstream functions of this protein. The aim of the present work is to investigate the acetylation status of NAT1 in human breast cancer cells. Breast cancer cell lines MDA-MB-231 (ER-, PR-, HER2-) and ZR-75-1 (ER+, PR+, HER2+) were cultured in the presence of HDAC inhibitor Vorinostat (SAHA) or Sirtuin inhibitor Sirtinol. Under these conditions, NAT1 protein, gene expression as well as enzymatic activity were quantified. Acetylation of NAT1 protein was evaluated following an immunoprecipitation and acetyl-lysine quantification. Finally, Sirt1 and Sirt2 genes were silenced using siRNA and NAT1 protein, gene expression and catalytic activity were quantified. Data was analyzed using one-way ANOVA with Tukey post hoc test, p < 0.05 was considered significant. The treatment of MDA-MB-231 or ZR-75-1 cells with increasing concentrations of SAHA (0.01-100 μM) resulted in 2 to 6-fold increase in NAT1 message expression (p<0.01); similarly, the NAT1 protein expression increased 2 to 3-fold (p<0.05) in both cell lines. Finally, the catalytic activity of NAT1 in the presence of SAHA increased 2-fold (p<0.05). Conversely, the chemical inhibition of Sirtuin activity produced decreases in the message, protein and catalytic activity, however, these changes were not statistically significant (p>0.05). Acetylated lysine in NAT1 was increased in the overnight cultured cells (p<0.01) and Sirtuin inhibited cells (p<0.05). Finally, silencing of Sirt1 and Sirt2 genes with siRNA resulted in reduced NAT1 protein expression (p<0.01) and NAT1 catalytic activity (p<0.01) but not NAT1 mRNA expression (p>0.05) These results provide evidence that chemical inhibition of HDAC enhanced lysine acetylation of NAT1 and its catalytic activity. Chemical and molecular inhibition of SIRT resulted in decreased NAT1 expression and activity in both breast cancer cells.

**2014 Ethylene Thiourea (ETU): Liver Mechanistic Study in B6C3F1 Female Mice**

S. Ganesan1, P. Munkerji2, J. Lagonell3, A. Venkatraman2, K. Johnson4, J. Domoradzki5, R. Settivari1, M. Aggarwal1, and C. Terry2.* Biomarker genes (unpublished data) and endpoints such as cellular vacuolation (≥100 ppm), increased mitotic figures (≥330 ppm), and intracellular edema (≥1000 ppm) groups, respectively, compared to control. ETU increased hepatocellular vacuolation in female mice only. In a previous mouse mechanistic study, ETU increased liver weight or food consumption were observed in any dose group. The study design included diets containing 0, 100, 330, and 1000 ppm of ETU for two days. No effects of body weight or food consumption were observed in any dose group. The study concluded that ETU slightly increased hepatic GSH (nonprotein sulfhydryl) concentration 24 to 36% in B6C3F1 mice without 6 to 6 hours following oral gavage administration. The objective of the present study was to further evaluate potential carcinogenic modes of action (MoA) in female mice. To test this hypothesis, key events (nuclear receptor activation determined by Cyp biomarker gene induction and an increase in hepatic acetylation), associative events (an increase in liver weight and hepatocyte hypertrophy), and toxicogenomics were evaluated. Four groups of adult female mice (10/group) were administered diets containing 0, 100, 330, and 1000 ppm of ETU for two days. No effects on body weight, food consumption were observed in any dose group. The overall mean daily intake of ETU was 15.52, 152.5 mg/kg/day. Relative liver weights were increased by 9%, 22%, and 26% in the 100, 330, and 1000 ppm groups, respectively, compared to control. ETU increased hepatic acetylation (≥100 ppm), increased mitotic figures (≥330 ppm), and increased hepatocyte cell proliferation (≥330 ppm) compared to the control group. RNAseq data showed a modest transcriptional response at 100 ppm ETU (56 genes) and a more robust transcriptional response at 330 (421 genes) and 1000 ppm ETU (539 genes). Both qRT-PCR and RNAseq data showed a minimal (~2-fold) difference in expression of nuclear receptor activation biomarker genes Cyp1a1, Cyp2b10, Cyp3a11, and Cyp4a10 at all dose levels suggesting no activation of nuclear receptors (AHR, CAR, PXR, and PPARα). Enriched canonical pathway analysis found activated oxidative stress-related pathways (NRF2- and glutathione-mediated oxidative stress) occurred in all ETU-exposed mouse livers. In conclusion, ETU induced transcript levels of antioxidants-related pathways suggesting oxidative stress may have contributed to the hepatic carcinogenic activity observed in the 2-year mouse study. The study was supported the Manczaceb Task Force.

**2016 Profiling Chemicals as Drivers of Breast Cancer Disparities by Integrating In Silico NHANES Biomarker Data with Chemical Activity Data from ToxCast and In Vitro Dose-Response Assessments**


Breast cancer mortality rates for non-Hispanic Black (NH) women are 40% higher than non-Hispanic white (NHW) women in the US. The mechanisms driving these differences are poorly understood. Here, we aimed to identify chemical exposures which play a role in breast cancer disparities. We integrated chemical biomonitoring data from the National Health and Nutrition Examination Survey (NHANES), biological activity data from the EPA’s Toxcast program, and in vitro dose-response assessments of cellular perturbations using the “Cell Painting” assay in MCF10A breast cells. Cell Painting is a high content imaging-based readout assay which uses 6 fluorescent stains to quantify cellular components including DNA, RNA, endoplasmic reticulum, mitochondria, f-actin, plasma membrane, and golgi apparatus, yielding approximately 3000 morphological measurements at the single cell level. Resulting data were analyzed with BMDExpress software. In NHANES, a total of 45 out of 143 chemicals had significantly higher biomarker concentrations in NHW women. Investigation of these chemicals in ToxCast resulted in 5371 assays for analysis. Median biomarker concentrations of propylparaben, methylparaben, 2,5-dichlorophenol, and p,p’-DDE (0.17μM, 3.1μM, 0.1μM, and 0.32μM respectively) in NHANES participants showed activity in multiple assays of breast cancer related genes in ToxCast. Frequently upregulated genes include ESR1, AR, PGR, POU2F1, XBPI, SMAD1, TP53 and MYC. Benchmark dose analyses of Cell Painting data from MCF10A cells dosed with 7 nM - 50 μM BPA identified morphological changes at human exposure relevant 7 nM, as low as 3 nM BPA, including alterations in actin, RNA and golgi staining intensity and granularity. In aggregate, these results show that NHW women are disproportionately exposed to a suite of chemicals which are biologically active at levels relevant to human exposure. Our long-term goal is to categorize chemical effects into functional pathways, using biological signatures to identify the chemical factors driving breast cancer disparities.

**2017 Pesticides and Other Chemicals That Increase Estradiol or Progesterone Synthesis May Increase Breast Cancer Risk**

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Established breast cancer risk factors, such as hormone replacement therapy and reproductive history, are thought to act by increasing estrogen and progesterone (P4). The most relevant carcinogenic active chemicals has focused on chemicals that bind to and activate the estrogen receptor, little attention has been paid to chemicals that may affect steroidogenesis, for example by increasing synthesis of estradiol (E2) or P4 systemically or in the breast. Using EPA’s ToxCast, 182 chemicals that increased estradiol (E2-up) and 186 that increased progesterone (P4-up) were identified from among 2,012 chemicals tested in an in vitro high-throughput steroidogenesis assay using human H295R adrenocorticaloma cells. Active chemicals were prioritized by potency and efficacy using the lowest effective concentration and the maximum fold change as proxies. Of 45 chemicals that were tested in the H295R assay and previously reported to cause mammary tumors or other mammary effects, based on information from sources such as the International Agency for Research on Cancer and the National Toxicology Program, 29 chemicals increased E2 or P4 synthesis, including the well-known mammalian gland carcinogen DMBA. A literature review was also conducted to compile breast cancer relevant in vivo data for the top 10 most efficacious and/or potent E2-up or P4-up chemicals. In many cases the mammary gland was not adequately evaluated or effects were dismissed. Chemicals whose...
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not cause DNA damage in intestinal tissue. High-throughput screening (HTS) were not measured in intestinal tissue, and it has been shown that Cr(VI) does dysregulation of DNA repair genes has been demonstrated, epigenetic effects already represented in the two proposed MOAs. For example, while epigenetic was no evidence of any underlying mechanisms or key events that are not al-

in vivo, oral exposure, gastrointestinal tissue). were screened for these four KCCs and further prioritized based on relevance mechanistic data represented in the existing two MOAs were produced regenerative cell proliferation. However, concerns persist that all possi-
tumors in mice have been proposed, with the latter involving cytotoxicity-in-

Hexavalent chromium [Cr(VI)] has long been recognized as a lung carcinogen towards -acetylation of carcinogens such as ABP and BNA.

alleles. Based on these results, we conclude that acetyl CoA affinity differs signifi-
cantly lower than (p< 0.05) although both are slow acetylator variant m values were 3541 ± 1120, 4823 ± 5232 and 1276 ± 349 µM, respectively. Acetyl CoA Km value of NAT2*7B was significa-
cantly lower than NAT2*5B (p< 0.05) although both are slow acetylator variant alleles. Based on these results, we conclude that acetyl CoA affinity differs towards NAT1 and NAT2 as well as between reference and variant alleles towards N-acetylation of carcinogens such as ABP and BNA.

Acetyl CoA Km for NAT2*4 (reference) and Variant Alleles.

N-acetyltransferases or NATs are polymorphic xenobiotic metabolizing en-
zymes catalyzing acetyl coenzyme A (acetyl CoA) dependent acetylation of many carcinogens. First, acetyl CoA binds to NATs and then the acetyl group is transferred to carcinogen leading to formation of the acetylated product. 4-aminobiphenyl (ABP) and Beta-naphthylamine (BNA) are aromatic amine carcinogens found in cigarette smoke, hair dyes and linked to urinary bladder cancer. ABP can be N-acetylated by both NAT1 and NAT2. To understand the relative overall importance of NAT CoA in these carcinogens en-
coded by different NAT1 and NAT2 alleles, we used Chinese hamster ovary (CHO) cells stably transfected with human NAT1*4 (reference), NAT1*14B (variant), NAT2*4 (reference), NAT2*5B (variant) or NAT2*7B (variant). In vitro N-acetyltransferase assays towards ABP or BNA were done using cell lysates and different acetyl CoA concentrations (1.25 - 5000 µM). The N-acetylated products were identified and measured using HPLC. Kinetic parameters for acetyl CoA were calculated using the Michaelis- Menten equation. For ABP, the apparent acetyl CoA Km for NAT2*4 was significantly higher (17 -fold) than that of NAT1*4 (p <0.001). The apparent acetyl CoA Km values were 95.1 ± 26.5 µM and 1615 ±124.8 µM for human NAT1*4 and NAT2*4, respectively, reflecting higher affinity of acetyl CoA towards NAT1*4. Acetyl CoA Km values were 1118 ± 420 and 785 ± 315 µM for NAT2*5B and NAT2*7B, respectively and both were significantly lower (p<0.001) than NAT2*4. For BNA, NAT2*4, NAT2*5B and NAT2*7B acetyl CoA Km values were 3541 ± 120, 4823 ± 5232 and 1276 ± 349 µM, respectively. Acetyl CoA Km value of NAT2*7B was signifi-
cantly lower than NAT2*5B (p< 0.05) although both are slow acetylator variant alleles. Based on these results, we conclude that acetyl CoA affinity differs between NAT1 and NAT2 as well as between reference and variant alleles towards N-acetylation of carcinogens such as ABP and BNA.

2019 Assessment of Mechanistic Data for Hexavalent Chromium-Induced Rodent Intestinal Cancer Using the Key Characteristics of Carcinogens

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Hexavalent chromium (Cr(VI)) has long been recognized as a lung carcinogen via inhalation exposure, and more recently recognized as an oral carcinogen in rodents exposed to very high concentrations in drinking water. Mutagenic and non-mutagenic modes of action (MOAs) for Cr(VI)-induced small intestine tumors in mice have been proposed, with the latter involving cytotoxicity-induced regenerative cell proliferation. However, concerns persist that all possible MOAs have not been fully considered. To address the potential for alternative MOAs, mechanistic data not represented in the existing two MOAs were evaluated. Relevant data were identified and organized by key characteristics of carcinogens (KCCs); literature related to epigenetics, immunosuppression, receptor-mediated effects, and immortalization were reviewed to identify po-
tential key events associated with alternative MOAs. Over 200 references were screened for these four KCCs and further prioritized based on relevance to the research objective (i.e., in vivo, oral exposure, gastrointestinal tissue). Minimal data were available specific to the intestine for these KCCs, and there was no evidence of any underlying mechanisms or key events that are not already represented in the two proposed MOAs. For example, while epigenetic dysregulation of DNA repair genes has been demonstrated, epigenetic effects were not measured in intestinal tissue, and it has been shown that Cr(VI) does not cause DNA damage in intestinal tissue. High-throughput screening (HTS) data related to the KCCs were also evaluated, with activity generally limited to assay endpoints related to the two recognized MOAs. The results of the review demonstrate that there is not strong evidence to support alternative MOAs (or key events) other than those that have already been proposed for Cr(VI) small intestine tumors and utilized by regulatory bodies globally.

2020 The Key Characteristics of Carcinogens

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The key characteristics of carcinogens (KCs) are the essential properties of car-
inecogenic agents. They are based on empirical observations of the chemical and biological properties associated with of the diverse agents classified as “carcinogenic to humans” (Group 1) by the International Agency for Research on Cancer (IARC). The KCs concept, which originated at an expert workshop convened by IARC, has been applied in IARC Monographs evaluations of more than 50 diverse chemicals and complex exposures suspected to cause cancer since 2015. The KCs are now used as the basis of a uniform approach to the evaluation of mechanistic data by IARC, strengthening the evidence in-
tegration approach in 2019 amendments to the IARC Monographs Preamble. This presentation will provide an overview of the mechanistic conclusions concerning the KCs in recent IARC Monographs classifications. The KCs pro-
vided an agnostic and unbiased survey of the mechanistic literature and were applicable to the systematic evaluation of a broad range of potential cancer hazards in vivo and in vitro. As such, the KCs improved uniformity across eval-
uations of mechanistically diverse agents, enhancing transparency, revealing strengths as well as gaps in evidence, and improving consideration of mech-
anistic similarities and differences across agents. However, some challenges were also identified. Across KCs, there was variability regarding the extent of testing, the availability of assays and biomarkers, and specificity for carcino-
genic (versus toxic) effects. This complicated interpretation of evidence on individual KCs. By leveraging interrelationships among the KCs, these limita-
tions can be addressed in future evaluations even while new assays and bio-
markers in different test systems are under development. Overall, this work will aid application of novel molecular research findings to identify the causes of human cancer, the first step in cancer prevention.

2021 Accounting for Multiple Comparisons in Statistical Analysis of the Extensive Bioassay Data on Glyphosate

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Glyphosate is a widely used herbicide worldwide. In 2015, the International Agency for Research on Cancer (IARC) reviewed glyphosate cancer bioassays and human studies and concluded that the evidence for carcinogenicity of glyphosate is sufficient in experimental animals and that glyphosate is probably carcinogenic to humans. We analyzed 10 glyphosate rodent bioassays, including those in which IARC found evidence of carcinogenicity, using a multi-response permutation procedure that adjusts for the large number of tumors eligible for statistical testing and provides valid false-positive probabilities (i.e., valid p values). The test statistics for these permutation tests are functions of p values from a standard test for dose-response trend applied to each specific type of tumor in each individual study. We evaluated 3 such permutation tests, using as test statistics the smallest p value from a standard statistical test for dose-response trend and the number of such tests for which the p value is ≤ .05 or .01. The false-positive probabilities obtained from 2 im-
nulations of these 3 permutation tests are: smallest p value: .26, .17; # p values ≤ .05: .08, .12; and # p values ≤ .01: .06, .08. In addition, we found more evidence for negative dose-response trends than positive. Thus, we found no strong evidence that glyphosate is an animal carcinogen. The main cause for the discrepancy between IARC’s finding and ours appears to be that IARC did not account for the large number of tumor responses analyzed and the increased likelihood that several of these would show statistical significance simply by chance. This work provides a more comprehensive analysis of the animal carcinogenicity data for this important herbicide than previously available. These results support our analysis of the human data on glyphosate which found that the evidence for the carcinogenicity of glyphosate in hu-
mans is consistent with being due to recall bias in the case control studies.
Prescription opioids are powerful pain-reducing medications, but they also can have serious side effects, especially when being misused/abused/overdosed. Thousands of articles that focus on prescription opioid use (POU) and its associated medical disorders have been published. As a preliminary study to systematically understand the risk factors related with the increased POU-associated medical disorders, we applied the well-adapted topic modelling method, Latent Dirichlet Allocation (LDA), to perform text mining on POU-related published literatures. We have collected six large academic abstract datasets by searching PubMed using Mesh words as prescription opioid, codeine, morphine, hydrocodone, oxycodone, and methadone. We then applied topic modeling to identify topics and analyze topic similarities/differences in these six datasets. Word clouds and histogram were used to depict the distribution of vocabularies over each topic, where the most prevalent words conveyed a topic’s meaning. We found that the LDA topics recaptured the search keywords in PubMed, and further revealed relevant themes, such as patients, drugs, side effects, and association links between different POU and risk factors, such as sex and age. Moreover, based on topic modeling’s results, TreeMap were used to fingerprint abstracts, which shows the possibility of making visualized literature index by combining topic modeling and visualization tools such as TreeMap. In addition, the trend analysis was performed to explore the hot topic dynamics in POU-related literatures, and the increasing trend in opioid prescription and its associated health risks were assessed as hottest issues.

Chemical epidemiology studies (CES) that allege adverse outcomes are effectvily alleging a toxic response. Such allegations can either lead to meaningful regulatory action or untoward consequences. In all cases, it is imperative that such allegations be independently critically reviewed by focused experts with access to all relevant supporting data. Currently, CES are reviewed by the separate regulatory agencies and accepted at their face value and it is difficult to obtain supporting data critical for independent review. Thus, decisions on the significance of CES may vary from agency to agency. In order to better serve the public, it was previously proposed (2020, Toxicology 177(1):156) that a more centralized “Panel” be established that would organize standards for the conduction, execution and submission factors that would be uniform for all regulatory agencies as well as systematically review CES. The proposal is an evolving concept and the current version presented here more clearly calls for establishing a fully independent self-supporting with permanent staffing entity. The proposed entity would consist of six subcommittees as i. ethics, ii. endpoint evaluation, iii. statistics, iv. exposure, v. chemical analysis and vi. animal toxicity and SAR each independently providing assessments for their respective disciplines. The ethics subcommittee would be largely responsible for developing standardized criteria for the design, conduction and reporting with an emphasis on developing means to obtain relevant data in a manner that protects the privacy of all subjects. The endpoint subcommittee would be experts on the nature of the adverse outcome and provide input on its etiology, known causes for its variation including genetic and any known chemicals, and the size of a cohort necessary to make a meaningful statistical difference. It will also maintain a compendium of chemicals known to be uniquely toxic to humans or where CES have demonstrated adverse outcomes in human studies. Finally, the chemical’s Cautions or Harmful effects the entity would produce a report that will articulate its conclusions as to whether there is a sufficient basis to verify the alleged adverse outcome or identify significant study deficiencies. Concerned parties may appeal the conclusions but only with supporting scientific data. The aim of the proposed entity is to benefit of the public, the toxicology and epidemiology communities, industry and public interest groups.

Occupational Exposure to Cleaning Products and Their Association with Respiratory Diseases: A Review

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Scientific and lay publications have raised questions regarding occupational exposure to cleaning products and the incidence of respiratory disease. A literature review was conducted to evaluate the association between work-
from tissue-embedded shrapnel fragments have not been fully elucidated. The goal of this study was to investigate the impact of having an embedded metal fragment on a Veteran’s overall health. 9,000 Veterans from the VA Toxic Embedded Fragment Registry, which uses self-reported data to identify Veterans at risk for embedded fragments, were randomly selected to receive a questionnaire which asked about the nature of their injuries, military exposure history, and current health concerns. This study included the Veterans RAND 12 (VR-12) item Health Survey, a well-validated instrument assessing overall physical and mental health. Using responses to injury-focused questions, Veterans were categorized into a high or low risk group based on likelihood of having an embedded fragment. Of 2,007 respondents who reported a blast or a blast injury, the most recent Veterans who were identified to be at high risk and 44% (22.1%) at low risk. Using a standardized VR-12 scoring algorithm, the overall study population mean Physical Component Summary (PCS) and Mental Component Summary (MCS) scores and standard deviations (SD) were 55.6 (SD 10.3) and 36.4 (SD 13.8), respectively. Compared to mean population norms of 50 (SD 10), these findings suggest poorer physical and mental health in this Veteran cohort. A generalized linear model was used to investigate if Veterans at high risk for having an embedded metal fragment had worse physical and mental health scores compared to those at low risk while controlling for age, race, smoking status, military branch, and injury severity. No statistically significant difference in PCS scores was found between the two risk groups. However, MCS scores were significantly greater in the high versus low group (p<0.005), indicating better mental health status in Veterans at high risk for having embedded metal fragments. This suggests the need for future research on the effects of embedded metals on long-term health, and whether other factors, such as health services access, could further explain these results.

2027 Contribution of Demolition Activities to Flint, Michigan, Childhood Blood Lead Levels in 2013-2015


Children residing in close proximity to demolitions of old residential or commercial structures may experience increased blood lead levels (BLLs) due to the release of debris containing lead-based paint. This analysis sought to investigate the potential influence of demolition activities that took place during the time period of 2013 to 2015 on the BLLs of children that resided in Flint, Michigan. Between 2013 and 2015, approximately 1,104 residential structures were demolished throughout the nine wards in Flint, where over 95% of the housing stock was built prior to the lead paint ban. Ward-specific mean BLLs (aggregated over April 2014-October 2015) and % BLLs elevated above the CDC reference level (%EBLL) (aggregated over January-September 2015) were compared to the number of demolitions by ward. Ward 5 had the most demolitions as well as both the highest mean BLL and %EBLL. The Mean BLL was significantly correlated to the number of demolitions by ward (R²= 0.75; p=0.02), while %EBLL was not significantly correlated (R²=0.42; p=0.27). Demolition activities that took place in Flint, Michigan from 2013 to 2015 may explain inconsistencies between the reported water lead levels and BLLs of children during the Flint Water Crisis (FWC). Specifically, it has been reported that BLLs were significantly increased in 2015, while water lead levels peaked in 2014 and returned to pre-FWC levels by 2015. In addition, a recent study found that the 2015 increase in percentage of Flint children with EBLL was confined to the third quarter, which aligns with expected seasonal increases in soil and dust exposure rather than ongoing consumption of tap water. Based on the results of this analysis, demolition activities in areas of downtown Flint with a high density of blighted structures may help explain the 2015 BLL peaks.

2028 Multi-metal Analysis of Private Well Water in North Carolina: Implications for Exposure Assessment and Public Health

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44.5 million people nationwide and 2.4 million people in North Carolina (NC) use private wells as their primary drinking source. Despite this, well water quality is not regulated under the Safe Drinking Water Act, thus well water users are vulnerable to metal contamination. Chronic exposure to metals is a global and national public health concern. Inorganic arsenic, cadmium, lead and mercury are all listed in the Agency for Toxic Substances and Disease Registry’s list of top ten hazardous substances and are known developmental toxicants. There are, however, few detailed, systematic documentations of domestic well water-based exposure. To address this gap, in this study, we set out to comprehensively examine statewide trends in both toxic and essential metals, arsenic, cadmium, lead, mercury, chromium, manganese, cop-

2029 The Association of Fluoride Exposure with Insulin Resistance and Prediabetes in US Children and Adolescents

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The primary route of fluoride exposure is drinking water, and previous studies have shown associations of fluoride with negative health effects. However, there are few epidemiological studies of its effect on the risk of diabetes in children. In this study, a cross-sectional study was conducted to investigate the association between fluoride exposure and insulin resistance and prediabetes. Children and adolescents were from the National Health and Nutrition Examination Survey (2013-2016) for a total of 5720 subjects who were aged 6 to 19 years and had plasma fluoride level measurements. The multivariate logistic regression model was fitted to estimate the odds ratio, after adjusting for demographic, socioeconomic, and other risk factors. The plasma fluoride levels were categorized into quartiles. The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated to identify subjects with insulin resistance. The status of diabetes was classified based on the diagnosis, and the levels of hemoglobin A1C, fasting plasma glucose, and 2-hour oral glucose tolerance test. In additional analyses, the fluoride levels were included as a log2-transformed continuous variable. The odds ratio for the insulin resistance was 2.17 (95% CI 1.30, 3.60) in the highest quartile, compared to the lowest quartile of plasma fluoride level, and a significant dose-response linear relationship was observed between quartiles of fluoride and insulin resistance (p-trend <0.01). The risk of insulin resistance was elevated significantly with each doubling of plasma fluoride levels (OR 1.35, 95% CI 1.04, 1.75). In addition, a significant dose-response linear relationship with quartiles of fluoride and prediabetes (p-trend <0.01). For plasma fluoride level as a continuous variable, each doubling of plasma fluoride levels was associated significantly with an elevated risk of prediabetes reporting per doubling of fluoride level. Our study suggests that fluoride may increase the risk of insulin resistance and prediabetes in children and adolescents. Further prospective studies are required to assure our findings.

2030 Electronic Cigarette (E-cig) Vapor Increases Brain Senescence and Mimics Idiopathic Pulmonary Fibrosis (IPF) Responses

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Aging exponentially increases the mortality rate and incidence of numerous diseases, including IPF and Alzheimer’s disease (AD). Aging can be partially attributed to the accumulation of senescent cells. These cells acquire a senescence-associated secretory phenotype (SASP), releasing proinflammatory markers into the extracellular milieu. The senescence-associated transcriptomes have been shown to be species-, tissue-, and stimulus-dependent. However, a large body of evidence points to the accumulation of senescent cells as underpinning these disease states. Although great advancements have been made in the field of senescence, the ability of electronic cigarette (e-cig) vapor to transform healthy cells into a senescent state has been largely unexplored. Initial reports showed that e-cig vapor can cause senescent-promoting events in a COPD mouse model, and as the popularity of e-cigs grows within American youth culture, this suggestive link warrants thorough investigation. As such, we exposed C57BL/6 mice to e-cig vapor (propylene glycol plus vegetable glycerin, with or without % phytol) daily for 2 months to de-
termine brain and lung senescence responses. Exposures were 2h/d for 5d/week as an average concentration of approximately 125 mg/m³. We quantified senescence markers within the prefrontal cortex through RT-qPCR, and performed proteomic analysis on the bronchoalveolar lavage fluid (BALF). Our data show statistically significant increases in inflammatory and senescence markers (e.g. p21, p16, IL-6) within the prefrontal cortex, and a striking overlap in expression when compared to published human PB-PF BALF proteomic dataset. Specifically, 10 gene ontology terms that encompassed cellular oxidant detoxification, nicotinamide nucleotide metabolic process, and hydrogen peroxide catalytic processing, among others. These initial data illustrate the imperative need to further explore the causal link between e-cigarette vapor and senescence transformation.

2031 Individual Chemical Exposure to Environmental Contaminants in Harris County, TX, from Baseline to Post-Hurricane Harvey Flooding

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Over 200,000 homes were damaged or destroyed when the Houston area in Harris County, Texas experienced extreme rainfall and catastrophic flooding from Hurricane Harvey. Importantly, 13 Superfund sites and several chemical/petroleum facilities were involved in unplanned chemical releases into the environment due to facility shutdowns and infrastructure damage. As clean-up efforts began, individuals and communities immediately raised questions regarding the human health impact of possible increased chemical exposure resulting from the hurricane and subsequent flooding. Oregon State University’s Food Safety and Environmental Stewardship (FSES) program formed a multi-institutional team with expertise relevant to disaster research response which included Baylor College of Medicine (BCM) and University of Texas-Houston School of Public Health (UT-SPH). Together we deployed personal sampling devises in the form of silicone wristbands to a longitudinal cohort of individuals (n=99) immediately after the hurricane and again one year later. We used the second time point from one year after the hurricane as a baseline chemical exposure. When comparing the two time points, we were able to evaluate whether Hurricane Harvey impacted chemical exposure to the longitudinal participants of our study, and more broadly to individuals living within the impacted area. Using Gas Chromatography-Mass Spectroscopy, we analyzed each wristband for 1,530 chemicals. The total detections of chemicals from the wristbands from the Hurricane Harvey study were further compared to other wristband studies conducted by FSES within the United States using the same analytical method. The mean chemicals detected/wristband was higher in the two sampling periods surrounding Hurricane Harvey than any other wristband study conducted by FSES within the U.S. using the same analytical method. When comparing across the two time points in Harris County, TX it was found that one hundred forty eight chemicals were detected at higher concentrations post flood compared to only forty chemicals that were detected at higher concentrations at the estimated baseline. Initial analysis indicates that chemicals classes detected in higher frequency immediately after the hurricane were polycyclic aromatic hydrocarbons (PAHs) and flame retardants were detected in higher frequency immediately after the hurricane, while pesticides were detected in higher frequency one year after the hurricane. Additionally, while unique differences in exposure profiles exist between the two time points, most of the chemicals detected were common at both time points (n=130) suggesting similar point sources.

2032 Pesticide Exposure Levels in the US and Their Bioactivity

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Pesticides are heavily used in agriculture globally and are potentially harmful to human health. Using 2 large publicly available datasets, this study aims to 1) quantify differences in pesticide exposure levels of the US general population in comparison to farmworkers, and 2) assess biological activity of these pesticides at human relevant doses using ToxCast high throughput screening toxicology data. The National Health and Nutrition Examination Survey (NHANES) is a cross-sectional study representative of the US population with oversampling weights for minority populations. NHANES was used to quantify pesticide exposure among US farmworkers and the general population who responded to NHANES between 1999 and 2016. Overall, there are 65,893 the NHANES survey with occupation data available with at least one pesticide biomarker present. Biomarker measurements were available for 28 different pesticides, which also have been assessed for dose-dependent toxicity using high throughput assays in ToxCast. Of the 28 pesticides, o,p’-DDT had the lowest average biomarker concentration at 0.03µM among US farmworkers, whereas p,p’-DDE had the highest exposure level at 0.5µM. In ToxCast, the AC50 represents the concentration at 50% of the maximum biological activity. The average AC50 for p,p’-DDE is 1.3µM suggesting that activation levels are higher that of the US population exposure level range. Ongoing work includes pesticide biomarker integrations into the ToxCast platform, including new occupational and demographic groups with biologically active concentrations based on high throughput toxicity data. By comparing population exposure data to toxicological assay data, our goal is to create an overarching view of how pesticides may be affecting the body at a human population level.

2033 Improving the Inhalation Cancer Risk Assessment for Hexavalent Chromium: Lung Cancer Mortality and Exposure Reconstruction in Aircraft Manufacturing Workers with Long-Term Low-Level Exposures

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For decades, lung cancer risk assessment for hexavalent chromium (Cr(VI)) has been based on historic occupational epidemiology studies of male chrome production workers exposed for relatively short durations to extremely high concentrations, and particularly toxic forms, of Cr(VI). Uncertainties related to using these data to assess cancer risk from low level environmental and occupational exposures are numerous, and improved epidemiologic data and robust risk assessment methods are needed to better quantify risk associated with chronic exposures in the low concentration range. To address this need, the Cr(VI) exposures and updated mortality experience of 7,233 aircraft manufacturing workers, including 715 women, with average plant tenure of 19 years, were studied over a 60-year period (1960-2019). Among 4,892 dece- dents, 347 lung cancer deaths were observed vs. 336.1 expected, resulting in a Standardized Mortality Ratio of 1.03 (95% CI 0.93-1.15). Using a Bayesian model, individual-level cumulative Cr(VI) exposures were calculated from in- dustrial hygiene data collected from the workplace and from other aerospace industry operations with similar exposure scenarios. Median airborne Cr(VI) concentrations, by job category for aircraft assembly, abrasive blasting, sand- ing, painting, and electroplating ranged from 0.29 µg/m³ to 6.6 µg/m³. The mortality follow-up and exposure reconstruction results will be combined for future dose-response analyses, employing Bayesian modeling approaches, and including key explanatory variables.

2034 Maternal Levels of Perfluoroalkyl Substances (PFAS) during Early Pregnancy in Relation to Preeclampsia Subtypes and Biomarkers of Preeclampsia Risk

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Prenatal exposure to perfluoroalkyl substances (PFAS) has been previously associated with preeclampsia, although findings are mixed. To date, no study has examined associations between PFAS and preeclampsia subtypes, which may have distinct etiologies. Therefore, we examined associations between PFAS and preeclampsia, including individual preeclampsia subtypes (i.e. early- and late-onset), in a case-control study (n=105 cases, n=75 controls) nested in the prospective LIFECOES birth cohort. In addition, we es- timated the association between PFAS and maternal angiogenic biomarkers (i.e. soluble fms-like tyrosine kinase-1 [sFlt-1] and placental growth factor [PLGF]). Biomarkers of PFAS exposure were quantified in maternal plasma collected during early pregnancy (median 10 weeks gestation) and angiogenic biomarkers were quantified in maternal plasma from up to four study visits (median 10, 18, 26, and 35 weeks gestation). First, we estimated the odds ratios (OR) and 95% confidence intervals (95% CI) associated with an interquartile range (IQR)-increase in PFAS and preeclampsia using logistic regression models. Second, we estimated the percent change (95% CI) in angiogenic biomarker concentrations associated with an IQR-increase in
PFAS using linear regression models. Lastly, in a mixtures-based approach, we estimated the joint associations between PFAS and both preeclampsia and angionic biomarkers using quanitle g-computation. After adjusting for confounders, both perfluorooctanoic acid (PFDA; OR: 1.76, 95% CI: 1.07, 2.91) and perfluorooctanesulfonic acid (PFOS; OR: 2.29, 95% CI: 1.21, 4.33) were associated with higher odds of late-onset preeclampsia, though associations were null for overall and early-onset preeclampsia. Similarly, a simultaneous one-quartile increase in PFAS was associated with higher odds of late-onset preeclampsia (OR: 2.28, 95% CI: 1.12, 4.64) in our quanitle g-computation models. We observed few associations between PFAS and angionic biomarkers. Heterogeneity of preeclampsia should be considered in future studies as populations may have different distributions of disease subtypes.

2035 Addressing the Activity of Binary and Complex Environmental Polychlorinated Biphenyl Mixtures toward the Dopamine Transporter

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When neurons experience a change in dopamine availability it can cause symptoms of addiction, attention deficit hyperactivity disorder (ADHD), and Parkinson’s disease. Polychlorinated biphenyls (PCBs) congeners are man-made environmental pollutants disrupt the dopamine transporter (DAT) that is responsible for dopamine uptake from the synaptic cleft. When DAT is altered it may lead to altered cognitive proficiency. To date 46 congeners in the ortho position that were highly chlorinated PCB congeners were found to alter DAT activity and include NDL PCB congeners 95. The binding assay inhibitory concentrations (IC₅₀) from this work and that of others will then be used to develop and apply a neurotoxic equivalency scheme (NEQ) to published PCB mixture concentration to predict DAT activity. Preliminary applications of a ternary and complex environmentally mixtures towards DAT using radioligand binding assay in female rat synaptosomes. Preliminary data confirms the inhibitory activity of NDL PCB congener PCB 95. The binding assay inhibitory concentrations (IC₅₀) was found that the serum of children in East Chicago had a DAD NEQ of 10.73 nanograms per gram. The ability of PCBs to induce DAT inhibition and alter dopamine concentrations can aid in addressing its harmful persistent neural activity during neurodevelopment and its effect on increasing neurodegenerative disorders especially Parkinson’s disease. Supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number R25GM071638.

2036 Variation of Extraction Technique on Fine Particulate Matter (PM₁₀₋₂.₅) Filters: Chemical and Toxicological Analysis

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Exposure to fine particulate matter (PM₁₀₋₂.₅) exacerbates a number of cardiovascular health effects, such as cancers of the lungs or bronchial system, asthma, and heart disease. To better understand the health effects of PM₁₀₋₂.₅ chemical and toxicological analyses are performed, a process that frequently requires collection of PM₁₀₋₂.₅ onto filters with extraction from the filter prior to analysis. However, there is not a standardized method for PM₁₀₋₂.₅ extraction. Different methods exist between groups, with variation in solvents and practices. In this study, four different filter extraction techniques common in toxicology research were used to determine if the extraction method impacts the resulting chemical composition and oxidative potential. Co-located PM₁₀₋₂.₅ filters (n=8) from four locations were collected by the Arkansas Department of Environmental Quality on January 4, 2012. Each filter was split into equal quadrants, each undergoing one of four extraction methods (sonication in acetone 1:1) in order to provide hydrophilic and hydrophobic fractions of the samples. The extracts were assessed regarding their endocrine disrupting potential using a battery of endocrine disrupting genes involved in metabolism, immunity, and development; suppresses immune function; impacts endocrine signaling; and decreases reproductive success in exposed aquatic populations. We previously demonstrated the generalized concentration addition (GCA) model most accurately predicts in vitro responses of mixtures of two GR ligands. Contrary to the concentration addition (CA) model, the GCA model accounts for <100% maximal efficacy of partial agonists. Previously, the model was applied to mixtures containing a single partial agonist; therefore, we aim to apply the GCA model to mixtures of multiple partial GR agonists and compare the model predictions to CA model predictions and observed in vitro responses. We used natural and synthetic glucocorticoids commonly detected in surface water and wastewater including dexamethasone, fluticasone propionate, triamcinolone acetonide, clobetasol propionate, prednisolone, 21-hydroxyprogesterone, corticosterone, and aldosterone. Using in vitro observed EC₅₀ Hill slope, relative potency factor, and maximum efficiency values, we applied GCA and CA models to four equipotent and non-equipotent tertiary mixtures of full and partial agonists. All mixtures were modeled at half-log concentrations. Equimolar mixture results were graphed with GraphPad Prism 7.00 while non-equipotent mixtures were modeled using an 8x8 factorial matrix design and graphed with SigmaPlot 14.0. GCA modeled responses predict chemical mixtures containing a lower concentration of a DAT NEQ based on the highly potent PCB 110, found that the serum of children in East Chicago had a DAD NEQ of 10.73 nanograms per gram. The ability of PCBs to induce DAT inhibition and alter dopamine concentrations can aid in addressing its harmful persistent neural activity during neurodevelopment and its effect on increasing neurodegenerative disorders especially Parkinson’s disease. Supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number R25GM071638.

2037 Application of the Generalized Concentration Addition Model to Predict In Vitro Responses of Tertiary Mixtures of Glucocorticoid Ligands

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Glucocorticoid receptor (GR) ligands, both endogenous and pharmaceutical, are frequently detected in the environment and exhibit high potency at low concentrations. Prolonged exposure alters expression of protein encoding genes involved in metabolism, immunity, and development; suppresses immune function; impacts endocrine signaling; and decreases reproductive success in exposed aquatic populations. We previously demonstrated the generalized concentration addition (GCA) model most accurately predicts in vitro responses of mixtures of two GR ligands. Contrary to the concentration addition (CA) model, the GCA model accounts for <100% maximal efficacy of partial agonists. Previously, the model was applied to mixtures containing a single partial agonist; therefore, we aim to apply the GCA model to mixtures of multiple partial GR agonists and compare the model predictions to CA model predictions and observed in vitro responses. We used natural and synthetic glucocorticoids commonly detected in surface water and wastewater including dexamethasone, fluticasone propionate, triamcinolone acetonide, clobetasol propionate, prednisolone, 21-hydroxyprogesterone, corticosterone, and aldosterone. Using in vitro observed EC₅₀ Hill slope, relative potency factor, and maximum efficiency values, we applied GCA and CA models to four equipotent and non-equipotent tertiary mixtures of full and partial agonists. All mixtures were modeled at half-log concentrations. Equimolar mixture results were graphed with GraphPad Prism 7.00 while non-equipotent mixtures were modeled using an 8x8 factorial matrix design and graphed with SigmaPlot 14.0. GCA modeled responses predict chemical mixtures containing a lower concentration of a DAT NEQ based on the highly potent PCB 110, found that the serum of children in East Chicago had a DAD NEQ of 10.73 nanograms per gram. The ability of PCBs to induce DAT inhibition and alter dopamine concentrations can aid in addressing its harmful persistent neural activity during neurodevelopment and its effect on increasing neurodegenerative disorders especially Parkinson’s disease. Supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number R25GM071638.

2038 Endocrine-Disrupting Potential of Complex Pollutant Mixtures in Indoor Dust Samples Assessed by In Vitro Bioassays


The indoor environment can be an important reservoir for a number of chemicals, many of which can become associated with dust. These chemicals can be released from building materials, appliances, furniture or introduced following the use of insecticides or household products. Once present on the surfaces they can be taken up through ingestion, inhalation, or dermal absorption which might cause adverse outcomes to the physiology of those exposed. The present work investigated the endocrine disrupting potential of mixtures of pollutants associated with dust samples from seven different indoor types. The sampling was done by vacuuming in old and new houses, cars, kindergartens, offices, public spaces and schools. The bulk dust was extracted by ultrasonication using two different solvents (methanol and hexane: acetone 1:1) in order to provide hydrophilic and hydrophobic fractions of the samples. The extracts were assessed regarding their endocrine disrupting potential using a battery of in vitro assays focused on several important mechanisms of action. Reporter-gene human cell-based assays revealed the interactions of studied samples with thyroid hormone receptor, estrogen and androgen receptors (both agonism and antagonism) as well as aryl hydrocarbon receptor. The results showed that both polar and non-polar fractions of indoor dust from every sampled microenvironment can interfere with signaling of these receptors, which are important in many physiological functions, namely endocrine regulation. The results from bioassays are being linked with extensive chemical analyses to prioritize the toxic drivers present in the samples and potentially contributing to the observed effects. Considering that people spend about 90% of their life in indoor environments, the chronic exposure to indoor pollutant mixtures with endocrine disrupting potential might be as-
sociated with adverse health impacts. Moreover, the elucidation of the effect drivers can represent an important step in the risk assessment of the human exposure. Supported by the grant 19-204795 from Grant Agency of the Czech Republic.

2039 Testing the Efficacy of Broad-Acting Sorbents for Environmental Mixtures Using Isothermal Analysis, Mammalian Cells, and H. vulgaris

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The hazards associated with frequent exposure to polycyclic aromatic hydrocarbons (PAHs), pesticides, Aroclors, plasticizers, and mycotoxins are well established. Adsorption strategies have been proposed for the remediation of soil and water, although few have focused on the mitigation of mixtures. This study tested a hypothesis that broad-acting sorbents can be developed for diverse chemical mixtures. Adsorption of common and hazardous chemicals was characterized using isothermal analysis from Langmuir and Freundlich equations. The most effective sorbents included medical-grade activated carbon (AC), parent montmorillonite clay, acid-processed montmorillonite (APM), and nutrient-amended montmorillonite clays. Next, we tested the ability of broad-acting sorbents to prevent cytotoxicity of class-specific mixtures using 3 mammalian in vitro models (HLF, ESD3, and 3T3 cell lines) and the hydra assay. AC showed the highest efficacy for mitigating pesticides, plasticizers, PAHs, and mycotoxins. Clays, such as APM, were effective against pesticides, Aroclors, and mycotoxins, while amended clays were most effective against plasticizers. Finally, a sorbent mixture was shown to be broadly active. These results are supported by the high correlation coefficients for the Langmuir model with high capacity, affinity, and free energy, as well as the significance of the standard cells and hydra IC50. The hydra:hydrazine:pyrazine levels in 3T3 cells and hydra showed the highest correlation as suggested by both Pearson and Spearman (p < 0.0001). Collectively, these studies showed that broad-acting sorbents may be effective in preventing toxic effects of chemical mixtures and provided information on the most effective sorbents based on adsorption isotherms, and in vitro and aquatic organism test methods.

2040 Predicting the Activation of the Androgen Receptor by Complex Mixtures of Environmental Antagonists Using Generalized Concentration Addition

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Nuclear receptors are ligand-activated transcription factors and are the mechanism by which lipophilic hormones and hormone-like molecules regulate gene expression. Thus, they are critical targets of endocrine disrupting chemicals. The androgen receptor (AR) is a nuclear receptor that binds ligands including hormones (e.g., testosterone), pharmaceuticals (e.g., flutamide) and environmental endocrine disrupting chemicals (e.g., cypermethrin, vinclozolin, procyominde). The vast majority of environmental AR ligands are antagonists. We developed Generalized Concentration Addition (GCA) to be able to predict the effect of mixtures of ligands with varying efficacies. 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. We previously developed a pharmacodynamic model for this system that meets the mathematical requirements for GCA and showed that it predicts the activation of AR by binary ligand mixtures. Here, we tested the efficacy of GCA in predicting the effect of complex mixtures of environmental ligands on AR activation. We generated individual dose-response data for BPA, BPC, DDE, HPTE, methoxychlor, procyominde and vinclozolin using the MDA-kb2 AR reporter cell line. Because these ligands are competitive antagonists, dose responses were determined in the presence of the full agonist BM5364929, and binding affinities were estimated using a Schild analysis. We also compared kinetics and assay particulars for values reported in Tox21/EDSP21. A mixture was designed with equipotent concentrations of the chemicals, and we used the reporter assay to assess AR activation by the mixture using a ray design. Empirical results were well predicted by GCA. We currently are investigating the ability of GCA to predict the effect of mixtures on endogenous target gene expression (PSA) in a human prostate cancer model (VCap cells).

2041 Construction and Application of the GC-MS/MS Database for the Aroma Component Analysis in Tobacco


Tobacco aroma components are very important to the aroma and taste of tobacco products and greatly influence the quality, characteristics and acceptability of the finished product. The matrix of tobacco is very complex and most aroma components are low threshold and in trace level. Gas chromatography tandem mass spectrometry (GC-MS/MS) with dynamic multiple reactions monitoring (dMRM) mode could provide a sensitive and selective gain to a broad range of target compounds. The database for tobacco aroma components provided information on the most effective sorbents based on adsorption isotherms, and in vitro and aquatic organism test methods. Finally, a total of 610 aroma component standards were analyzed by GC-MS/MS. Full scan mass spectrum and product ion spectra at eight different collision energies (CE) were obtained for each target compound, and at least four groups of recommended ion pairs and corresponding CE were selected for each aroma component. Secondly, the retention indices of 610 aroma components under programmed temperature conditions were detected based on the semi-nanopolar chromatographic column represented by DB-SMS UI, and the retention indices of 163 components had not been recorded in NIST 17 database yet. Retention indices of the targets were used to predict the retention times of the target compounds under different chromatographic conditions, which could be further used to accurately predict the acquisition time window in multi-analyte GC-MS/MS method rapidly. Adding retention index also successfully promoted the accuracy and reliability of qualitative analysis among isomers. Thirdly, this study proposed a strategy for the rapid establishment of GC-MS/MS quantitative analytical methods: 1) Establish a qualitative analytical method based on the ion pairs and corresponding CE information in the database mentioned above and the predicted retention time calculated by retention indices. 2) Select aroma components which were detected in the real samples and need to be analyzed. 3) Establish a quantitative analytical method for the selected targets. Finally, a quantitative analytical method for 207 aroma components in tobacco was established based on the GC-MS/MS database, and 20 tobacco samples were detected, which demonstrated that this GC-MS/MS database could be applied to the analysis of aroma components in tobacco.

2042 Non-targeted Analysis (NTA) of Flavors in Aged E-liquid Formulations: Case Study

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e-Liquid formulations are typically composed of carriers (propylene glycol [PG], vegetable glycerin [VG]), nicotine, and varying levels of flavor mixtures. Prior to preclinical toxicological assessment of e-liquid and e-vapor inhalation studies, test material characterization and stability evaluation are critical to confirm the composition is consistent throughout the testing period. As part of pre-work for a long-term inhalation study, we first investigated the stability of prototype e-liquid formulations containing flavor mixtures (18% w/w) for a short-term stability evaluation. The formulations were sealed in amber glass vials with minimal headspace and analyzed after storing under refrigerated and ambient conditions for the duration of the study. It was determined that the formulation with nicotine was stable for 3 days, whereas the formulation without nicotine was stable for up to 10 days. Therefore, use of the materials were limited to these maximum lengths of time during the course of the study. Secondary, the aged formulations were subjected to non-targeted analysis (NTA) to identify any byproducts after exaggerated long term storage up to 2 years. In general, regardless of the presence of nicotine, the majority of the flavor compounds were stable for ambient storage conditions for 2 years. For the flavors that did not meet the study stability criteria (>80% target concentration), byproducts were identified from NTA: for example, low molecular weight flavor compounds (e.g., 1-penten-3-one), sulfur containing compounds (e.g., p-mentha-8-thiol-3-one) and compounds containing ester functionality (e.g., eugenyl acetate). In summary, test materials such as e-liquid formulations may change over time and intended ingredients, as well as unintended byproducts, should be characterized based on study design. This is critical for study planning and accurate interpretation of “dose-response” assessments of biological testing.
Appropriate functioning of the hypothalamic-pituitary-gonadal (HPG) axis is critical to maintaining a healthy pregnancy. During pregnancy, estradiol concentrations steadily rise to levels 100 to 1000 fold greater than the normal estrous cycle. The drastic endocrine changes of pregnancy may create a critical window vulnerable to perturbation by endocrine disrupting chemicals (EDCs). Understanding HPG axis disruption during pregnancy is critical, because it is possible that the disruption of estradiol can have important effects on developing metabolic disease, including gestational diabetes. Multiple EDCs studied individually have been shown to have anti-estrogenic properties. Our previous research indicates exposure to a mixture of EDCs induced unique metabolic dysfunction in pregnant mice, which was not seen for single EDC exposures. However, it was unknown if this effect is specific to pregnancy. To assess the effect of exposure to multiple EDCs on endocrine and metabolic health during pregnancy, pregnant dams and non-pregnant control females were exposed to a mixture of EDCs (MIX) from a variety of chemical classes, at-ratine (10mg/kg), bisphenol-A (50µg/kg), perfluorooctanoic acid (0.1 mg/kg), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (0.036µg/kg). Pregnant dams were exposed to MIX from gestational day (GD) 0.5 to GD16.5 and non-pregnant females were concurrently exposed to MIX for the same acute exposure duration. Pregnant MIX exposed dams had lower concentrations of serum estradiol at GD16.5. Only pregnant MIX exposed dams had hyperglycemia in a glucose tolerance test at GD16.5, indicative of gestational diabetes. Additionally, pregnant MIX dams had a greater gestational weight gain than vehicle exposed pregnant dams, as well as a greater weight of visceral fat. These data provide biological plausibility for the epidemiological associations observed between estradiol concentrations during pregnancy and increased risk of developing gestational diabetes. Additional studies were performed to elucidate the pregnancy-specific mechanisms of metabolic reprogramming (elevated blood glucose and adipose). In conclusion, a mixture of anti-estrogenic EDCs during pregnancy can lower estradiol levels and disrupt maternal metabolic function, increasing blood glucose and visceral fat, effects not seen in non-pregnant female mice. Supported by P30 ES001247 and T32 ES007026.

Pregnancy Is a Critical Window for Endocrine-Disrupting Chemical Effects on Maternal Endocrine and Metabolic Health
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Organophosphate (OP) toxicity can occur as a result of exposure from a variety of household and occupational sources (i.e. field workers, military, etc.). Each year, 3 million people are exposed to OPs, contributing to approximately 300,000 deaths. Previous high-exposure OP research has shown the activation of MAPK signaling pathways depending on the type of OP and brain region. These same populations experience stress at an increased rate relative to the general population. Thus, corticosterone (CORT) has been used to stimulate this response associated with high stress that would also likely be experienced during OP exposures. Previous results have demonstrated this combination increases neuroinflammation, and Miller et al., recently demonstrated spatial differences in acetylcholine accumulation after exposure to chlorpyrifos oxon (CPO) and disopropyl fluorophosphates (DFP). Therefore, an investigation into the phosphoprotein responses in the cortex were performed to better understand the dissimilarities of these early cellular changes involved with these OPs and their co-exposure to CORT. Using a validated mouse model, adult male C57BL/6J mice were exposed to CORT in the drinking water for 7 days followed by a single injection of DFP (4.0 mg/kg, i.p.) or CPO (8.0 mg/kg, i.p.) on day 8 and euthanized 30 min, 2 h, and 24 h post-injection via focused microwave irradiation. To evaluate brain-region-specific effects, 12 post-translationally modified protein targets were measured using a multiplex ELISA using four dosing regimens. Several phosphoprotein responses (RPS6, CREB, and JNK) were found to be significantly increased (p<0.05) for CORT+CPO exposure, but not for CORT+DFP. Conversely, SMAD2 was significantly phosphorylated for DFP exposures relative to CPO. Moreover, quadratic discriminant analysis of the significant phosphoproteins was found to predict the temporal tissue response to each exposure with little error. This supports the notion that neuroinflammatory response in this mouse model may be driven by off-target mechanisms of AchE exposure. Further investigation of other relevant exposures and their phosphoprotein signaling effects must be performed to better understand potential biomolecular drivers of OP toxicity.

Organophosphate Pesticides with Corticosterone Priming Elicit Disparate Phosphoprotein Signaling in a Mouse Cortex
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Traditional safety assessment involves evaluation of single chemicals rather than chemical mixtures. However, realistic exposure involves multiple chemicals at various exposure levels. Additionally, research has shown that mixtures have different toxicological profiles than their individual components. Current whole mixture safety assessment involves whole mixture extracts and are timely and expensive. Sufficienly similar mixtures are another form of whole mixture safety assessment which uses existing toxicological data from a surrogate mixture. Currently, there is a lack of an established framework to form sufficiently similar mixtures, from environmental exposure data, to conduct a safety assessment of a contaminated site. In this study, several approaches are explored to form sufficiently similar mixtures. Air sampling from a legacy creosote site will be the “real-world mixture”, and Coal Tar Extract (CTE), a well-studied complex mixture will serve as the “test mixture.” Existing toxicity values and potential for exposure were incorporated to establish an overall rank to identify drivers of toxicity in the whole mixture. Representative mixtures of the test and real-world mixture were formed based on relative abundance (ExpoMix), average toxicity values (AVG-ToxMix), and a weighted approach incorporating toxicity and abundance (WAVG-ToxMix). When compared to the whole mixture, the WAVG-ToxMix most effectively captured both high abundance and high hazard chemicals and was hypothesized to be most sufficiently similar to the whole mixture. Hazard characterization of these mixtures and the test mixture using the ExpoMix and AVG-ToxMix were developed using biomarkers of xenobiotic metabolism, oxidative stress, tight-junction integrity, and DNA damage. Apical endpoints for oxidative stress, tight-junction integrity, and cytotoxicity were also measured. AVG-ToxMix was a biomarker when compared to Mix (q<0.05) than ExpoMix. This confirms that less abundant, more potent, chemicals are driving toxicity and that hazard should be incorporated when prioritizing chemicals for representative mixtures. Future directions involve evaluating mixtures using in vivo endpoints in zebrafish, and comparing benchmark dose responses of ExpoMix, AVG-ToxMix, and WAVG-ToxMix to their respective whole mixtures to evaluate for sufficient similarity. Results from this study will help provide regulatory agencies with an experimental framework that can be used in whole mixture safety assessment.

A Novel Framework to Form Sufficiently Similar Mixtures from Environmental Exposure Data
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Breast cancer remains the most common cancer among women worldwide. Numerous studies associate lifetime risk with environmental exposures to endocrine disrupting chemicals (EDCs). Individual EDCs like bisphenol A have been shown to increase the risk of mammary cancer in rodents. However, epidemiological studies examining the relationship between breast cancer and other EDCs like perfluoroctane sulfonic acid (PFOS) have found inconclusive evidence. Some studies suggest that more complex chemical mixtures (or the exposure - the totality of exposures) could have a cumulative impact on breast cancer risk. The goal of this work is to characterize the evidence concerning environmental chemical mixtures. After a thorough literature search using terms related to breast cancer, chemical mixtures, and environmental exposures in PubMed, Scopus, and Web of Science, studies were categorized by the following routes of exposure: air pollution, consumed/food exposure, occupational exposure, pre/perinatal exposures, pesticidal exposure, and urban exposure. Evidence was assessed based on the category type of the mixture. Although a total of thirty-four papers met inclusion criteria, there were limited defined mixtures associated with breast cancer risk excluding the topics of tobacco smoke and urban air pollution. Animal studies are an invaluable resource when studying health outcomes associated with environmental exposure, but only a few featured the use of a rodent model to study the impact of mixtures on breast cancer risk. There is a strong need for defined mixture exposure studies in rodents to better understand the cumulative effect of chemicals regarding breast cancer risk. Before that is possible, much more work is needed to understand the full range of chemical mixtures that contribute to breast cancer risk.

Characterizing Environmental Mixtures and Their Contribution to Breast Cancer Risk: State of the Evidence
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Electronic Nicotine Delivery Systems (ENDS) contain a wide variety of flavor ingredients. While most flavor ingredients used in today’s ENDS are ‘generally recognized as safe’ (GRAS) for oral consumption, there are insufficient data on their safety via inhalation. Considering the range of different flavors used in ENDS and the resulting flavor mixtures, it is highly impractical to test all possible flavor combinations for inhalation toxicity. In this study, we developed a structure-based approach where more than 200 flavors commonly used in ENDS were clustered in groups of structurally related compounds and a total of 38 Flavor Group Representatives (FGRs) were selected. We propose that a representative FGR mixture could then be tested both in vitro and in vivo and the data generated could support the toxicological assessment of all structurally-related individual flavors, based on the “read-across” concept that structurally-related compounds would have comparable metabolic and biological activities (as outlined in the European Commission Regulation (EC) No 1565/2000). Prior to FGR selection, toxicological predictions were made for key endpoints using the TOPKAT (e.g., pluritoxicity, pCarCinogenicity, pChronic LOAEL and pDevToxicity) and QSAR toolbox (e.g., pCramerClass) to fill gaps, if experimental data were not available in literature (e.g., LD50, ExpCarCinogenicity). In addition, we characterized experimentally the cytotoxic potential of more than 200 flavors by real-time cellular analysis (XCelligence) and a set of most cytotoxic substances was evaluated using High Content Screening (HCS). HCS data for the remaining flavors was obtained using a predictive model based on pCramer, pluritoxicity, pChronicLOAEL, ExpCarCinogenicity and XCelligence (pToxPHCS). Finally, flavors within each group were ranked based on: LD50, pDevToxicity, pToxPHCS, pChronicLOAEL and pluritoxicity scores, in order to select the predicted most toxic FGR for each structural group. This approach allowed the selection of FGRs that could be tested alone or in combination as mixtures to generate in vitro and in vivo data and to determine acceptable levels for their use in ENDS.

Concerns over livestock health issues that recently developed in cattle, equine and wildlife in the state of Florida, USA lead to questions surrounding the safety of pasture forages that those animals were consuming. A collaboration and wildlife in the state of Florida, USA lead to questions surrounding the safety of pasture forages that those animals were consuming. A collaboration and wildlife in the state of Florida, USA lead to questions surrounding the safety of pasture forages that those animals were consuming. A collaboration and wildlife in the state of Florida, USA lead to questions surrounding the safety of pasture forages that those animals were consuming. A collaboration and wildlife in the state of Florida, USA lead to questions surrounding the safety of pasture forages that those animals were consuming. A collaboration and wildlife in the state of Florida, USA lead to questions surrounding the safety of pasture forages that those animals were consuming. 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Dietary nitrates and nitrates have been associated with potential risks and health benefits, thus igniting a debate about the health outcomes of ingested nitrates and nitrates. The risk and benefit assessment of nitrates and nitrates is complicated due to the endogenous conversion of nitrates to nitrates within the human body. To address the issue, this study aimed to assess the risks and benefits of dietary nitrates and nitrates based on internal dose in a probabilistic manner by integrating exogenous exposure based on the total diet study with endogenous exposure using a human toxicokinetic (TK) model. The sampling design of this study was based on the core food approach of the Taiwan total diet study. The population TK model was performed using Monte Carlo simulation to predict internal doses of nitrates and nitrates for different age populations. The dietary risk related to nitrates and nitrates was estimated based on the acceptable daily intake (ADI). The internal dose levels of nitrates and nitrates associated with cardiovascular protective effects were collected from peer-reviewed literature. Nitrates levels in vegetables (range of means: 3.22 - 164.1 mg/kg) tend to be higher than those in fruits (2.23 - 88.06 mg/kg), rice (mean: 9.70 mg/kg), and processed meat (38.69 - 73.97 mg/kg). In conclusion, the combined CEDI for I-168 and its degradation products (5.42 mg/p/day) is below the ADI (60 mg/p/day); therefore, there is not a safety concern for its current use. Also, there is no evidence to suggest that the degradants of I-168 pose a special risk for neurotoxicity. This approach is deemed appropriate to evaluate the safety of I-168 when used as an AO in food contact applications.
Brown seaweeds (class Phaeophyceae) are a well-known source of polyphenols. Cystoseira baccata is an eastern Atlantic macroalgae species. The study objective was to investigate the potential cytotoxicity and the antioxidant activity of polyphenolic compounds extracted from C. baccata in Caco-2 cells. The total phenolic content extracted in aqueous media was 2.28 ± 0.03 mg fresh algae. The possible cytotoxic effect of the aqueous/phenolic extract on cell viability was measured by quantitative colorimetric MTT assay and on oxidative damage [reactive oxygen species (ROS) production and caspase-3 activity] was also evaluated. Exposure of the extracts aqueous/phenolic (10, 50, 100, 250, 500 µg/mL) evoked no changes in cell viability and caspase 3 activity. Exposure of the extract at concentrations of 250 and 500 µg/mL evoked a reduction of ROS production (15.5% and 20%, respectively) compared to control. Then, we investigated the potential protective effects of the extract against an oxidative stress induced by tert-butyl hydroperoxide (t-BOOH) in Caco-2 cells. Treatment with t-BOOH, significantly in a dose-dependent manner, evoked MITT reduction, and increase of ROS levels and caspase 3 activity. Treatment with the extract (100-500 µg/mL) entirely prevented the MTT reduction caused by t-BOOH and caused a significant reduction, dose-dependent, of the oxidative damage induced by t-BOOH. Increases in ROS generation (334%) and caspase-3 activity (41%) induced by t-BOOH were significantly reduced by 62 and 36%, respectively, when the cells were pretreated with the extract (100, 250 and 500 µg/mL). The results showed that treatment of Caco-2 in culture with the extract aqueous/phenolic from C. baccata confers a significant protection against the oxidative insult. These findings support the use of the extract aqueous/phenolic from C. baccata for potential nutraceutical purposes. Work supported by Project Ref. RTA2015-00010-C03-03 from Ministerio de Economía, Industria y Competitividad (Spain) and CERCA Programme, Generalitat de Catalunya (Spain).
cantly decreased compared to the NR BGLs at 60 minutes, although no significant alterations were observed for other time points. Meanwhile, ITT analysis revealed that the BGLs of EGP-SD were also significantly decreased compared to the NR BGLs at 120 minutes. When these results were compared to the previous EGP-FD study at two months, EGP-FD treated groups exhibited significantly lower BGLs at 15 minutes compared to the NR control, while ITT analysis showed no significant changes. Taken together, our results suggest that EGP-S MD may exhibit decreased insulin resistance and increased glucose metabolism to a higher degree than EGPs-FD. Supported by NIH R41DK121553.

2057 Evaluation of Mycotoxins in Florida Forages
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A wildlife conservation center in Florida has observed an intermittent staggering syndrome and death in their white rhinoceros (Ceratotherium simum simum) population. The initial presentation was acute death in multiple rhinoceroses. Later, staggering and other neurologic abnormalities were noted, with one case progressing to death. Bloodwork was overall normal, and necropsies of deceased animals revealed generally nonspecific lesions, but no clear indication of hepatic tissue damage, possibly by lipotoxicity. Histological details of tissues corroborated well with these observations and showed an increased incidence of microvesicles and hepatocyte ballooning in the TCO fed rats that was comparatively higher in the CO fed animals. The activities of serum hepatic enzymes (e.g., AST, ALT, and ALP) were more prominent in TCO fed animals. Thus, there was a clear indication of hepatic tissue damage, possibly by lipotoxicity. Histological details of tissues corroborated well with these observations and showed an increased incidence of microvesicles and hepatocyte ballooning in the TCO fed rats than in CO fed rats. Further, TCO fed animals showed alterations in their hepatic redox equilibrium. Hepatic GSH was elevated in both CO and TCO fed rats, while hepatic GSHPx activity was lower in TCO fed rats. This suggests that TCO feeding may have induced oxidative stress and oxidative damage in the hepatic tissue of rats.

2058 Zearalenone Metabolites: Study of In Vitro Enzymatic Defense in Undifferentiated SH-SYSY Cells and In Silico Metabolomic Prediction of Toxic Effects
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α-Zearalenol (α-ZEL) and β-zearalenol (β-ZEL) are mycotoxins produced by Fusarium species, both zearalenone metabolites (ZEA) and present in food. Once ingested, metabolite products can be generated by Phase I and II reactions and enzymatic defense activated. In vitro and in vivo studies associate effects of estrogenicity, oxidative stress, cytotoxicity and DNA damage to them. It is presented i) the evaluation of α-ZEL and β-ZEL on enzymatic glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD) and glutathione S-transferase (GST) activity in undifferentiated SH-SYSY cells, over 24h and 48h with individual and combination mixtures and ii) the metabolomics profile of ZEA’s derivatives predicting the toxic effect of 15 compounds by an in silico workflow using: MetaTox, SwissADME and PASS online programs. Up-regulation of the antioxidant defense system by increasing the activity of GPxs and SOD in both individual and mixture treatments after 24h and 48h, respectively was observed. A major reduction was detected in activity levels of CAT in both individual and mixture treatments after 24h. Similarly, this happened for GST after 24h (individual treatments) and 48h (mixture treatments), while an increased was noticed after 24h only for mixtures. In silico metabolomics profile for α-ZEL and β-ZEL came from products of O-glucuronidation, S-sulfation and hydrolysis; which had better properties to reach blood brain barrier than initial mycotoxins. Order of toxic effects with probability of activation was carcinogenic > nephrotoxic > hepatotoxic > endocrine disruption > mutagenic > genotoxic. Prediction of inhibition, induction and substrate function on different isoforms of Cytochrome P450 (CYP1A1, CYP1A2, CYP2C9, CYP2C9 and CYP3A4) varied for each compound and mycotoxin analyzed; similarly, for activation of caspases 3 and 8. This work was supported by the Spanish Ministry of Science and Innovation PID2019-108070RB-I00ALI and Generalitat Valenciana GV/PROMETO2018-126.

2059 High-Fat Diet Incorporated with Thermally Oxidized Coconut Oil Is More Detrimental Than Unoxidized Oil in Developing Steatohepatitis in Wistar Rats
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We evaluated coconut oil (CO) and thermally oxidized CO (TCO) as fat sources in the HFD/STZ-mediated type 2 diabetic rodent model for the development of non-alcoholic fatty liver disease (NAFLD). Female Wistar rats (6 in each group; av. bwt 200g) were fed a normal laboratory diet or HFD containing either CO or TCO for 2 months. STZ (30 mg/kg bwt) was administered intraperitoneally at the end of one-month feeding to induce diabetic changes. It was found that rats fed on the TCO-containing diet had increased serum levels of TC, TG, and VLDL compared to normal and CO-treated animals, indicating that TCO induced more pronounced dyslipidemia. At least 50% of the hepatic tissue and lipid accumulation in TCO or HFD fed rats were due to fatty infiltration. Higher fatty infiltration was seen in TCO fed rats. The lipid profiles of hepatic tissue showed increased PL and TG in the TCO fed rats while TC was comparatively higher in the CO fed animals. The activities of serum hepatic enzymes (e.g., AST, ALT, and ALP) were more prominent in TCO fed animals. Thus, there was a clear indication of hepatic tissue damage, possibly by lipotoxicity. Histological details of tissues corroborated well with these observations and showed an increased incidence of microvesicles and hepatocyte ballooning in the TCO fed rats than in CO fed animals. Taken together, the fatty liver-mediated lipidotoxicity and inflammation (hepatosteatosis) were more in TCO incorporated HFD than in CO-HFD diets. Further, HFD fed animals showed alterations in their hepatic redox equilibrium. Hepatic GSH was elevated in both CO and TCO fed groups compared to the normal group. The activities of glutathione peroxidase, glutathione S-transferase, catalase, and SOD were found to be increased. These were thought to constitute a protective mechanism against oxidative/carbonyl stress in the hepatic tissue of HFD rats. Supporting this, we found that CO and TCO-fed animals had enhanced lipid peroxidation (in-
creased TBARs). Thus, fatty liver with a heightened antioxidant defense, lipid peroxidation, and inflammation are all indicative of hepatosteatosis. Further, in support of this proposition, the hydroxyproline, an index of collagen formation, was significantly increased in TCO fed rats than in CO fed group. This was confirmed by the extent of procollagen in the hepatic tissue sections of TCO fed animals. These observations indicate that TCO induced hepatic damage was advanced to the level of fibrosis ignition. Overall, the study shows that formulation of HFD incorporated with TCO as a fat source in combination with STZ injection is an efficient dietary model for developing steatohepatitis with a fibrotic stage in rats within two-month time.

**2060 An Effect-Based Evaluation of the Presence of Hazardous Chemicals in Food Contact Materials Made from Paper and Cardboard**


Food contact materials (FCMs) can contain hazardous chemicals that may migrate into food and pose a health risk for humans. Previous studies and regulation are mainly focused on plastic materials, while data on packaging materials made from paper and cardboard are limited and regulation is lacking.

We used a strategy panel of cell-based bioassays to investigate the presence and impact of bioactive chemicals on several human relevant endpoints like oxidative stress, genotoxicity, inflammation, xenobiotic metabolism and endocrine effects in extracts made from paper and cardboard. In total, extracts of twenty three commonly used paper and cardboard FCMs on the Swedish market were tested at concentrations 0.3, 1, and 10 mg/mL. At the highest concentration bioactivities were observed in several of the samples: oxidative stress (52%), genotoxicity (100%), xenobiotic metabolism (74%) and antagonistic androgen (52%) and estrogen receptor (39%). Effects on xenobiotic metabolism were observed also at the lower concentrations. Packages of potential concern included cake/pastry boxes/mats, boxes for infant formula/kiddiemilk, pizza boxes, pizza slice trays and bag of cookies. Furthermore, packages that did not give any effects were paper for baking/baking moulds, board sausages and Fries. It can be hypothesised that the observed responses may be explained by inks, coatings, contaminants and/or naturally occurring compounds within the material. To summarise, an effect-based approach enables hazard identification of chemicals within FCMs, which is a valuable tool for ensuring safe use of FCMs.

**2061 N-acetyl-l-aspartate (NAA): Comparative PK Evaluation in Rat, Swine, and Goat following Single and Repeated Oral Dosing**

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N-acetyl-l-aspartate (NAA) is a modified amino acid present at low concentrations in many food contact materials. Oral exposure of rats to high NAA-doses (500 mg/kg/day) is associated with non-adverse acinar cell hyper trophy of the submandibular salivary gland. A multi-species comparative pharmacokinetic evaluation was conducted to evaluate systemic NAA exposure in rats, swine and goats at a pharmacologically-relevant estimated dietary intake of 25 mg/kg/day. Additionally, dose-proportionality of systemic NAA exposure in rats was evaluated over a range of physiologically- and toxicologically-relevant doses (10, 25, 75, 250 and 500 mg/kg/day). Groups of male and female rats, swine and goats were administered NAA orally for 14 consecutive days. Plasma samples were collected from each species and gender over a comparable time course on the first and last days of dosing. Samples were analyzed for NAA and L-aspartate concentration, and subjected to non-compartmental pharmacokinetic analysis. NAA was rapidly eliminated at each sampling interval and there was no evidence of accumulation or relevant differences in exposure between sexes of each respective species. In rats, Tmax values increased slightly with increasing dose, consistent with delayed absorption. Additionally, systemic exposure was greater than proportional at doses ≥ 250 mg/kg/day, suggesting saturation of clearance. At a common NAA dose (25 mg/kg/day), systemic exposures based on AUC(12)h values were comparable between rats and swine, while exposures in goats were much lower, suggesting a greater degree of pre-systemic metabolism in goats. Exposures in swine and goats at 25 mg/kg/day were 1-2 orders of magnitude below exposures in rats at the subchronic NOAEL of 500 mg/kg/day. Together, these results suggest that systemically-mediated effects observed at high-doses in rodents are unlikely to be reproduced in other species at doses consistent with physiologically-relevant estimated dietary intakes.

**2062 Safety Evaluation of DSM Bacillus subtilis Food Enzymes**

M. Galano, M. W. van den Dungen, T. van Rij, and H. E. Abbas. DSM Food Specialties B.V., Delft, Netherlands. Sponsor: M. Galano, EUROTOX

The safety of new microbially-derived food enzymes should be carefully assessed before their introduction on the market. The safety of any production strain is the key element of the assessment. DSM started its Bacillus subtilis strain lineage from a well-documented non-pathogenic and non-toxigenic Bacillus subtilis isolate and used strain improvement via a combination of random mutagenesis and targeted genetic modifications. As demonstrated in cytotoxicity studies, the introduction of strain modifications via either classical mutagenesis or genetic engineering did not lead to toxicity of the DSM Bacillus subtilis strain supernatants towards CHO epithelial cells, suggesting that none of the introduced mutations lead to the formation of toxic peptides with cytotoxic properties. Genome sequence analysis of the production strains confirmed the absence of vector sequences and of antibiotic resistance genes in the production strains. In addition, three food enzyme preparations produced by strains within this lineage did not show any genotoxic potential as shown in genotoxicity tests. 90-day rat oral toxicity studies performed with the same enzyme preparations did not reveal toxicological significant adverse effects, the No Observed Adverse Effect Level being assigned to the highest dose level tested in each study. These results further demonstrate absence of any safety concerns coming from the introduced genetic modifications.

Based on the establishment of the DSM Bacillus subtilis safe strain lineage, it is believed that enzymes produced by current and new strains derived from this lineage can be safely developed without performing additional genotoxicity and systemic toxicity studies, which will notably allow for reduction of the use of test animals.

**2063 Influence of Dietary Fatty Acid Biomarkers on Desaturase and Elongase Indices in Human Breast Adipose Tissue**


Individual studies observed positive associations of Δ9- and Δ6-desaturase indices (DIs) and negative associations of Δ5-DI and elongase index (ELOVLI) with type 2 diabetes (T2D). Furthermore, diet has been shown to affect DIs and ELOVLI in plasma indicating putative beneficial and adverse effects of diet on T2D. The influence of other lifestyle variables and of long term diet which is reflected in human adipose tissue (ADT) has not been investigated yet. Thus, the aim of this study was to identify variables influencing DIs and ELOVLI in human ADT. DIs were derived from pre- and postmenopausal women undergoing reduction mammoplasty (n=40). Fatty acid (FA) composition was analyzed by GC-FID. DIs and ELOVLI were calculated from product-to-precursor ratios. 7β-OH-cholesterol (7β-C), 5,6β-epoxy-ChOL (5,6β-C) and 5,6α-epoxy-ChOL (5,6α-C) as well as estrone (E1) and 17β-estradiol (E2) were determined by GC-MS/MS. Principal component (PC) analysis was applied for FAs yielding PCs reflecting diet rich in vegetable oils and nuts (VEG), fish (FISH) and hydrated vegetable oils (HYDR). 7β-C, 5,6β-C and 5,6α-C yielded PC OX, whereas E1 and E2 yielded PC ESTR. Multiple linear regression models were calculated using DIs and ELOVLI as dependent variables. PC VEG, FISH, HYDR, OX, ESTR, intake of estrogen-active drugs (EADs), menopausal status, age, pregnancies, smoking habits, alcohol consumption and BMI were used as explanatory variables. Notably, the adjusted coefficient of determination of the models was low (R²=0.30, 0.47, 0.18 and 0.30 for Δ9-, Δ6-, Δ5-DI and ELOVLI, respectively), suggesting important variables missing in the models. In accordance with studies investigating FA biomarkers in plasma, Δ9- and Δ6-DI were influenced negatively (p=0.006 and 0.0003) by VEG supporting a putative beneficial effect of VEG. Putative beneficial influence of BMI and intake of EADs was observed on ELOVLI (p=0.002) and Δ5-DI (p=0.004). Although FISH exhibited putative beneficial influence on ELOVLI (p=0.03), it also affected putatively adversely on Δ9-DI (p=0.03), suggesting an ambivalent role of FISH on DIs and ELOVLI. Alkie its association in plasma, smoking influenced Δ9-DI putatively adversely (p=0.04) in ADT as well. Likewise, the number of pregnancies influenced Δ6-DI putatively adversely (p=0.003). Interestingly, HYDR, OX and ESTR did not significantly influence any investigated index. In conclusion, diet and lifestyle not only influence DIs in plasma but also in human ADT.
The primary culinary practices that change the physio-chemical properties of the oils are thermal oxidation. The intake of such oils at higher percentages is known to have detrimental effects on health. Nevertheless, there is a gap in the available literature on the effect of consumption of these oils at normal dietary levels. The present study sought to explore the effects of prolonged consumption of thermally oxidized oils, primarily those consisting of long-chain saturated and polyunsaturated fatty acids (SFA and PUFA) on metabolic dysregulation pertinent to carbohydrate and lipid metabolism and associated inflammatory changes. Thirty male Wistar rats (av. b.wt 160-180 g) were randomly divided into 6 groups. Group I was kept as the control and fed a diet containing 5% groundnut oil as the fat source. Groups II and III were fed a normal rodent diet in which the fat source was replaced with 5% fresh and 5% thermally oxidized sunflower oil (SO and TSO; represents mainly long-chain PUFA) as the fat source. Groups IV and V were fed normal chow replaced with 5% fresh and 5% thermally oxidized palm oil (PO and TPO; represents mainly long-chain SFA) as the fat source. The feeding was carried over six months. Oral glucose tolerance test (OGTT) was performed in all groups at the beginning and the end of the experimental period. Body weights measured at weekly intervals. In addition to serum Lp-PLA2, and C-reactive protein (CRP), the activities of hepatic carbohydrate metabolizing enzymes such as fructose-1,6-bisphosphatase (F16BPase), succinate dehydrogenase (SDH), malate dehydrogenase (MDH), and isocitrate dehydrogenase (ISCDH) were measured. With regard to weight gain in different groups, it was found that rats fed on thermally oxidized oils failed to have gained adequate weight compared to their respective controls. Both SO and TSO animals had significantly higher Lp-PLA2 activity, however, CRP level was found to increase only in the TSO fed group. Serum total cholesterol was significantly lower in PO fed animals while LDL was significantly elevated in SO, TSO, and TPO fed groups. F1,6BPase activity was significantly reduced in SO and TSO groups. Hepatic SDH activity was also reduced significantly in both TPO and TSO groups. MDH activity, on the other hand, was significantly increased in PO and TSO fed animals. As a result of reduced OGTT in SO and TSO fed animals and the decreased activity of F1,6BPase (indication of inhibition of gluconeogenesis), the glycogen stores were thought to be lowered in these groups. Further, the reduced activity of hepatic mitochondrial MDH activity was thought to indicate reduced glucose oxidation. Together, the increased Lp-PLA2, in SO and TSO fed groups and increased CRP in TSO fed groups provide evidence for the induction of pro-inflammatory insults and dysregulated lipid profile. Prolonged consumption of thermally oxidized oil leads to the dysregulation of carbohydrate and lipid metabolism, subsequently resulting in an inflammatory micro-environment in the animals. It is thus possible that long-term consumption of deep-fried oils, even at lower quantities, may lead to degenerative diseases including CVD and cancer.
Environmental exposures contribute to the pathogenesis of lung fibrosis and we have demonstrated an increase in cadmium (Cd), a common component of cigarette smoke (CS) and environmental particulate matter (PM), in lung tissue from subjects with idiopathic pulmonary fibrosis (IPF). Cd activates peptidylarginine deiminase 2 (PAD2), a citrullination catalyzing enzyme, and induces a PAD2-dependent pulmonary fibrosis. Exposure to Cd also leads to the secretion of citrullinated vimentin (Cit-Vim) and Cit-Vim, but not vimentin, independently develops a similar pattern of fibrotic tissue remodeling in a TLR4-dependent manner. However, as a cytoplasmic protein, how does Cit-Vim translocate to membrane and secrete outside of the cells remains unknown. Protein disulfide-isomerase associated 3 (PDI3A), which construct thiol-disulfide interchanges in the endoplasmic reticulum (ER), mediates misfolded protein-induced apoptosis and associates with fibrotic mediators and airway hyperresponsiveness. Although ER stress has been involved in Cd-induced oxidative stress, whether ER stress involves in Cd-induced pulmonary fibrosis has not been elucidated. In this study, mice exposure to Cd in the presence or absence of phenylbutyric acid (PBA), an ER stress inhibitor, was used to evaluate lung fibrosis. Our results showed that Cd exposure activated macrophages and induced ER-stress marker proteins expression, including ER chaperone glucose-regulated protein (GRP) 78, CCAAT/enhancer-binding protein-homologous protein (CHOP), calreticulin (CRT) and PDI3A. PBA prevented Cd-induced Cit-Vim production, ER stress proteins accumulation and lung fibrosis. Compared to Vim, Cit-Vim bound highly to CRT and PDI3A and the binding affinities were significantly increased in the presence of Cd. We further demonstrated that PDI3A was required for Cd-induced Cit-Vim membrane localization. PDI3A deficiency using both PDI3A siRNA knockdown technique and specific inhibitor attenuated Cd-induced ER stress-related misfolded proteins accumulation, α-SMA activation, collagen deposition and cytokine/chemokine production. In conclusion, our results demonstrate that PDI3A mediates Cd-induced ER stress, Cit-Vim membrane localization, secretion, and subsequent lung fibrosis.

**2068** Protein Disulfide-Isomerase-Associated 3 (PDI3A) Is Required for Cadmium-Induced Citrullinated-Vimentin Membrane Localization, Secretion, and Pulmonary Fibrosis


**2069** Urinary Speciated Arsenic and HbA1c among US Adults

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Arsenic is a toxic metalloid that may be associated with diabetes. Few studies have investigated the association between urinary speciated arsenic and diabetes in humans. The objective of this work was to assess the association between urinary arsenic and diabetes among adults in the United States using the 2015-2016 National Health and Nutrition Examination Survey (NHANES) III data set. HbA1c (glycated hemoglobin/hemoglobin A1c) is a biomarker used to diagnose diabetes. We computed a categorical variable, diabetic status, with two categories: normal (HbA1c < 5.7%) and prediabetes/diabetes (HbA1c ≥ 5.7%). The exposure was total urinary arsenic and six different urinary speciated arsenic concentrations (arsenous acid, arsenic acid, arsenobetaine, arsenocholine, dimethylarsinic acid, and monomethylarsonic acid); these were dichotomized with 1 denoting a value greater than the lower limit of detection. We conducted chi-square tests of independence and ordinal logistic regression analysis using complex survey procedures. The sample consisted of 2,195 adults (20 years or older), of which 51.16% were females. Of the total sample, 29.99% had some college and 46.93% were married. In the ordinal logistic regression model, urinary arsenic acid (OR = 0.38, 95% CI = 0.17, 0.85), and urinary arsenocholine (OR = 1.68, 95% CI = 1.22, 2.31) were significant predictors to detect a difference between normal and prediabetes/diabetes. The study found a positive relationship between urinary arsenocholine and a borderline negative association with urinary arsenic acid with a level of HbA1c ≥ 5.7%.

**2070** A Physiologically Based Pharmacokinetic Model for Assessing Lifetime Cadmium Dietary Exposure with Implications for Risk Assessment


Cadmium (Cd) is a toxic metal found widely distributed in the environment. For the general population (i.e., non-smokers), dietary intake is the primary source of Cd exposure. Since Cd has a long half-life, Cd accumulates in humans through lifetime dietary exposure. Chronic exposure to Cd can cause several adverse effects, mainly kidney and bone damage. In order to assess the health risk during lifetime exposure to Cd, it is necessary to correlate lifetime Cd dietary exposure to the body burden of Cd. For this, a lifetime physiologically based pharmacokinetic (PBPK) modeling provides an effective platform to incorporate age-dependent changes in pharmacokinetics and dietary habits to evaluate their impact on internal exposure to environmental chemicals. The purpose of this study was to assess lifetime Cd dietary exposure from birth to the end of life using a human PBPK model and to apply the simulated results to dietary risk assessment. This study integrated the concentrations of Cd in 9 food categories in Taiwan (fish and seafood, algae and products, grains, poultry, meat, vegetables, fruits, beverages, and seasonings) obtained from the literature with the food consumption rates and body weights available from the Nutrition and Health Survey in Taiwan covering all ages to estimate the dietary intake of Cd. This study rebuilt and validated a previous human PBPK model and incorporated Monte Carlo simulations to perform population analysis for predicting Cd body burden due to dietary exposure over the lifetime. Our preliminary results found that algae and products (1.00 ± 1.35 μg/g), fish and seafood (1.19 ± 1.19 μg/g), and grains (0.04 ± 0.02 μg/g) contained the highest Cd levels in 9 food categories. The validation results showed that the rebuilt human PBPK model could reasonably predict the cumulative burden of Cd in urine due to dietary exposure to Cd throughout the lifetime. This population PBPK model provides a useful tool for lifetime exposure assessment in humans to aid food safety assessment.

**2071** A Tale of Two (Hg) Species: Intracellular Biotransformation of Methylmercury to Inorganic Hg\(^{2+}\) Elicits Greater Toxicity


Mercury (Hg) is an environmental toxicant of major concern to human health. One of the main routes of Hg exposure occurs via ingestion of methylmercury (MeHg) through consumption of fish and seafood. MeHg remains the most concerning form of Hg due to its widespread exposure and its effects as a neurotoxicant. Conventionally, the toxic mode of action following MeHg exposure has been attributed to MeHg directly. However, autopsy records from MeHg poisoning cases in humans, and studies in primates, suggest that MeHg is slowly biotransformed (demethylated) to inorganic Hg (iHg) at target organs. Therefore, we investigated the longstanding question of which species of Hg, MeHg or iHg, elicits greater toxicity intracellularly at target organs. The ability to study the effects of intracellular MeHg demethylation has been hampered by the lack of understanding of mechanisms of MeHg demethylation in eukaryotic cells. In this project we investigated the toxicologic impact of MeHg demethylation and resulting iHg intracellularly. To achieve this, we utilized the bacterial mer operon system which encodes the only known enzyme capable of MeHg demethylation, organomercurial lyase (merB). Using bacterial constructs, we expressed the merB genes from two distinct bacterial strains in isolation and in conjunction with a merA partner gene. By expressing merB in E. coli and assessing growth and cellular viability we see that production of iHg intracellularly exerts greater toxicity than exposure and uptake of equal concentrations of MeHg. To further investigate the consequence of intracellular demethylation in a eukaryotic model, we have turned to Drosophila, which lacks endogenous MeHg demethylation activity. Coding sequences of two bacterial strain variants of merB genes, K62 and RN23, were independently engineered for transgenic expression in recombinant Drosophila, allowing for spatiotemporal control via the Gal4-UAS system. Preliminary results demonstrate merB K62 RNA transcripts are appropriately expressed and translated, demonstrating MeHg demethylation in fly tissues. This Gal4-UAS system will be used to upregulate merB at target organs, allowing us to observe target organ specific toxicity associated with the generation of intracellular iHg. Understanding the consequences of MeHg demethylation will allow for a better understanding of the proximal toxic agent upon MeHg exposure. Supported by NIHES R01ES030940, P30ES001247, T32ES007026-43.
Northeastern British Columbia is known for intensive unconventional natural gas exploitation by hydraulic fracturing which can release contaminants like trace elements. Gestational exposure to these contaminants may lead to deleterious developmental effects. We aimed to assess pregnant women exposure to trace elements in the Peave River Valley study (EXPERIWA) provided daily spot urine samples over 7 consecutive days. Samples were analyzed for 20 trace elements. Median urinary levels were higher than those from reference populations for lithium (3 times), aluminum (3 times), gallium (2 times), strontium (2 times), and barium (2 times). The 95th percentile from reference populations was exceeded by many participants during the week for aluminum (35%), gallium (56%), strontium (55%), barium (56%), manganese (36%) and cobalt (31%). Our 50th and 95th vanadium levels were respectively at least 1.5 times and 2 times higher than those from reference populations. Other trace elements were similar or lower compared to those measured from reference populations. Moreover, we observed substantial within-person variability in urinary levels. For example, urinary barium levels varied over two orders of magnitude for some participants. Our results suggest that pregnant women from this region may be more exposed to certain trace elements (lithium, aluminum, gallium, strontium, barium, manganese, cobalt, vanadium) than reference populations and that a spot sample may be insufficient to adequately characterize exposure.

Arsenic is a ubiquitous occupational and environmental contaminant to which millions of people around the globe are exposed every day. It imposes threat to humans through its toxicity and carcinogenicity. However, some arsenicals are exploited for their remedial effects. Arsenic trioxide (ATO) is successfully applied in the treatment of acute promyelocytic leukemia (APL) and is currently under investigations for treating other types of cancer. Both inorganic arsenic and its methylated metabolites have been shown to modulate aryl hydrocarbon receptor (AhR)-regulated phase I and phase II xenobiotic metabolizing enzymes which are involved in the carcinogenic and cytoprotective pathways, respectively. Therefore, the aim of this study was to examine the impact of ATO on AhR phase I and phase II enzymes typified by cytochrome P450 1A1 (CYP1A1) and NADPH:quinone oxidoreductase 1 (NQO1), respectively. Human hepatoma (HepG2) cells were treated with ATO (1, 5 and 10 μM) in the absence or presence of 1 nM of the archetype AhR ligand, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). CYP1A1 and NQO1 expression was determined both at mRNA and protein levels using qPCR and Western blot analysis, respectively. MTT Assay was used to assess cell viability. Our results show that cell viability was not affected by all concentrations of ATO used. ATO alone didn’t confer changes in CYP1A1 mRNA and protein. However, ATO caused a concentration-dependent decrease of TCDD-mediated induction of CYP1A1 mRNA and protein levels. On the other hand, ATO, alone or in the presence of TCDD, significantly increased NQO1 at mRNA and protein levels. In conclusion, our study demonstrated that ATO differentially modulated the AhR-regulated CYP1A1 and NQO1 enzymes with subsequent implications on cellular response to xenobiotics co-exposure. However, further studies are required to explain the mechanism of this behavior. This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant RGPIN 250139 to A.O.S.E. M.A.E. is the recipient of Pharmacy PhD Alumni Graduate Student Scholarship.
The heavy metals arsenic (As) and lead (Pb) are environmental pollutants, often found in common sites, and linked to adverse health effects, including cardiovascular toxicity and neurotoxicity. A variety of studies have determined developmental toxicity and biomarkers of low dose exposure to each of these metals, but there are limited studies on the binary mixture. This study evaluated the interaction between As and Pb to determine what type of interaction occurs at lethal and sub-lethal concentrations using the zebrafish model. The concentration addition model was applied to survival data to determine if the interaction between Pb and As is additive due to shared neurotoxicity and vascular toxicity pathways. Metal exposures were from 1-hour post-fertilization (hpf) through 120 hpf. As concentrations were 0-1066 μM and Pb concentrations were 0-480 μM in the survival study. The LC25, LC50, and LC75 values at 120 hpf from the single metal exposures were used to determine the mixture concentrations for three separate mixture experiments. The survival data indicated an additive non-interaction effect at these concentration ranges. To further examine the impact of this metal mixture on development, behavior and morphological alterations were evaluated at sub-lethal concentrations of 10 and 100 ppb As (0.133, 1.33 μM) and lead (Pb) in the zebrafish (Danio rerio) at 14 dpf.
Lung cancer is a major cause of cancer death around the world and recovery rates have not improved for decades. A major factor in the lack of progress is that lung cancer is often missed at early stages that could be treatable due to smoking. Smoking cessation could potentially halve lung cancer incidence. This work was supported by the National Cancer Institute, the National Institute of Environmental Health Sciences [ES016893 to J.P.W].

Organotin compounds are compounds with one or more tin-carbon bonds. Triorganotin derivatives can be powerful fungicides and bactericides, depending on the organic group R, hence gaining the interest of many chemists. However, their applications are limited because of their poor water solubility. Increasing the solubility for some diphenyltin complexes has shown to increase their effectiveness so it is logical for scientists to conduct similar experiments with triorganotins. We hypothesized that ionic triorganotin derivatives will have improved solubility and biological properties as potential anticancer agents with reduced toxicity. Therefore, the goal of this research is to synthesize a triphenyltin complex with ionic characteristics. Synthesis of the ionic triphenyltin complex involved the reaction of a diprotic oxalic acid, which triphenyltin hydroxide (MeHg) and di-ethylamine. IR spectroscopy and X-ray Crystallography studies demonstrated that an ionic triphenyltin complex was successfully acquired. The ionic complex comprises of a triphenyltin cation and a polyoxoanionic anion. The formula of the complex is [(MeHg)₂(C₂O₄)₂]⁺[O₃P(C₆H₄)₂]⁻. The ionic complex was successful as a potential anticancer agent with reduced toxicity. However, the exact mechanism of action is unknown, and further studies are needed to elucidate its anticancer properties.

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MicroRNA (miRNA) are important regulators of gene expression that respond not only to developmental and pathological cues, but also to environmental stimuli. Dyslipidemia is a hallmark of metabolic conditions and has been shown to significantly affect the expression of circulating miRNA sequences. Recently our lab has shown that the environmental toxicant methylmercury (MeHg) causes dyslipidemia in the Caenorhabditis elegans model organism. While MeHg increases the expression of adiogenic transcription factors and lipid binding proteins in the worm, there is limited information on how the toxicant affects miRNA expression. We hypothesized that MeHg would increase the expression of adiogenic miRNA sequences and/or decrease the expression of anti-adiogenic miRNA sequences. We further hypothesized that the target miRNA sequences for the miRNAs affected by MeHg would also be altered. We selected three adiogenic (mir-34, mir-124, mir-355) and three anti-adiogenic (mir-240, mir-786, and let-7) miRNA sequences homologous to known human miRNA sequences altered in obesity, and quantified their levels 30 min, 24 h, and 48 h post MeHg treatment. In general, MeHg significantly increased miRNA expression 24 h post exposure for both adiogenic and anti-adiogenic sequences, however by 48 h only the adiogenic miRNA sequences were elevated, while the anti-adiogenic miRNA sequences were decreased. These data suggest that there are developmental changes in miRNA expression over time following MeHg exposure. We next selected one target miRNA sequence for each miRNA sequence based on miRNA-miRNA relationships observed in humans. While there were significant changes in gene expression for all miRNA sequences assayed, only the relationship between mir-240 and mir-786 with their shared target RNA paqr-2 showed a conserved relationship between nematode and humans. This data suggests the possible existence of target genes for mir-34, mir-355, mir-124, and let-7 that affect dyslipidemia in worms and man.

In Vitro Transport of Toxicants by MDR1 (ABCB1) Polymorphic Variants

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Multidrug resistance protein 1 (MDR1/ABCB1) mediates the cellular efflux of endogenous compounds and xenobiotics. Single nucleotide polymorphisms (SNPs) in the MDR1 gene can impact the disposition of drugs and toxicants, as well as influence disease susceptibility. In the present study, we sought to determine whether genetic variants in the MDR1 gene alter the cellular concentrations and toxicity of the chemotherapeutic drug doxorubicin and heavy metal cadmium. Human MDR1 SNPs (G1199A, C1236T, G2677T, and C3435T) were generated using site-directed mutagenesis and stably expressed in Flp-In 293 cells. Expression of MDR1 protein as well as the intracellular accumulation and cytotoxicity of doxorubicin and cadmium were quantified in cells expressing wild-type (WT) or polymorphic variants of MDR1. Compared to cells expressing an empty vector, overexpression of MDR1 WT cells reduced the intracellular accumulation and cytotoxicity of both toxicants. Cells expressing the variant G1199A had significantly increased total and cellular surface MDR1 protein expression (30-100%) compared to cells expressing MDR1 WT. Consistently, the intracellular accumulation of doxorubicin and cadmium was decreased by 20-50% in G1199A-expressing cells and in turn, conferred resistance to cellular apoptosis. Similarly, the G2677T variant decreased cadmium accumulation and apoptosis, likely due to enhanced efflux, compared to MDR1 WT. In contrast, cells expressing the C1236T variant had increased sensitivity to the toxicity of doxorubicin and cadmium without changing cellular concentrations. In summary, genetic variation in the MDR1 transporter can influence transporter activity and sensitivity to toxicants which may be important for identifying populations at heightened risk of chemical-induced injury. Supported by ES029275, ES035321, TR003017, and ES005022.
2089 Heavy Metal Contamination and Its Impact on a Native American Community: The Toxic Legacy Continues

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This community-engaged research project was initiated upon Tribal Nation request to address the extensive environmental contamination and associated health implications of the Rampagoua Lounega Nation. In the 1960s and 1970s, millions of gallons of paint sludge and other waste was dumped into abandoned mining areas, resulting in health problems for the Ramagoua Turtle Clan in Ringwood, NJ. This environmental devastation placed a huge burden on the Indigenous community who have lived on this land for generations. Using a citizen science approach, samples of soil, water, plants, and fish tissue were collected from control sites and Rampagoua residential areas outside the partially-remediated 500-acre Superfund site. Soil samples were collected around the community which were identified as zones 1 through 7. Each dried soil sample was analyzed twice in triplicate using two different XRF instruments and an ICP-OES. Additionally, water samples were collected at 1, 2, and 6 min from running faucets within a local church to test for the presence of heavy metals and analyzed using ICP-MS. Results revealed that both As and Pb concentrations in soil samples from zones 3, 4 and 5 exceeded the NJ Direct Contact Soil Standards and EPA Soil Screening limits of 19 and 400 mg/kg, respectively. Plants grown in distinct community neighborhoods also showed elevated levels of Zn and Cu above the WHO/FAO acceptable limit of 1.5 and 2 mg/kg, respectively. Additionally, Pb levels exceeded the national and federal standard of 15 ppb - were found in drinking water from several indoor church faucets. As Pb and As can cause acute and chronic illnesses, the results were quickly reported back to the community, the local governing body and media. These never-before identified contaminated drinking water findings led to a switch by the local Ramagoua Turtle Clan from a contaminated well source to the municipal water supply. This study demonstrates the importance of community-academic partnerships and citizen science for protecting the environmental health of marginalized and Indigenous populations. Supported by NYU NIEHS Core Center Pilot Program ES-000260-53.

2090 Inhibition of Rad18 by Arsenic

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Environmental exposure to arsenic enhances the carcinogenicity of ultraviolet radiation leading to increased risk of developing nonmelanoma skin cancers. The goal of this study is to elucidate mechanisms underlying the cocarcinogenic actions of arsenic to improve the health of the over 200 million individuals worldwide exposed to high levels of arsenic. Arsenite (AsIII) is known to bind and inhibit zinc finger (ZF) domains within DNA repair proteins, resulting in the retention of DNA damage. Accumulated DNA damage increases the burden on DNA damage tolerance pathways, the last line of defense against cell death or mutagenesis. In addition, unrepair DNA lesions can lead to DNA double strand breaks and chromosomal abnormalities. Rad18 is an essential factor to both DNA damage tolerance and double strand break repair mechanisms. It contains two functionally and structurally diverse ZF's, the RING finger and UBZ domain, which are structurally conserved in AsIII binding and disruption. Due to the increased propensity for mutagenesis, apoptosis, and chromosomal aberrations with AsIII exposure, the central hypothesis of this study is AsIII disrupts the function of Rad18. Preliminary results have shown zinc loss from endogenous Rad18 in AsIII treated neonatal Human Epidermal Keratinocytes (HEK), which suggests a loss of structure and function in one or both Rad18 ZF's. Mass spectrometry analysis of AsIII exposed Rad18 UBZ peptide revealed for the first time AsIII binding to the UBZ domain. There are only six proteins that contain the UBZ4-type motif found in Rad18 and all depend on the motif to maintain genome stability. Therefore, this novel discovery could have great implications on understanding arsenic carcinogenicity. HEK cells exposed to AsIII resulted in decreased PCNA and Rad18 monoubiquitination, indicating a loss of function of the Rad18 RING finger and UBZ domain, respectively. In addition to the structural and functional disruption of Rad18 ZF's, findings demonstrate a multifaceted effect of AsIII, including changes in Rad18 cellular localization. Alterations in Rad18 ZF function or regulation by AsIII can lead to genome instability and carcinogenesis via the loss of Rad18 dependent DNA damage tolerance and double strand break repair. Elucidating mechanisms supporting the link between environmental exposure to arsenic and the development of cancer will provide data pertinent to environmental policy decision making and the discovery of novel interventions.

2091 Arsenite Binds and Impedes ZNF598-Mediated Ribosome-Associated Protein Quality Control in Human Cells: Implication in Proteotoxic Stress

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Arsenic pollution in drinking water is a global public health problem, affecting the health of 200 million people in more than 70 countries. Many human diseases, including cancer and neurodegenerative diseases, stem from protein misfolding and/or loss of protein quality control during translation that affect cell homeostasis. Despite its well-known inhibitory role in DNA repair, arsenic's potential role in perturbing proteostasis is poorly understood. Here, we report that arsenite impaired ZNF598-mediated ribosome-associated protein quality control (RQC) by inhibiting ZNF598's E3 ubiquitin ligase activity, which highlights an emerging mechanism of arsenic-induced proteotoxic stress. Using arsenic-containing biotin and fluorescent probes, we demonstrated that arsenite interacts with the tetra-cysteine residues within the RING finger motif of ZNF598 in cells by competitively displacing its bound zinc ions. Our LC-MS/MS-based proteomic data revealed that GM00637 human skin fibroblast cells exposed to 5 μM arsenic for 24 hours down-regulated the site-specific ubiquitination levels of ZNF598-targeted lysine residues 138 and 139 on ribosomal proteins RPS10 and lysine 8 on RPS20 to 60% and 52% respectively, which are regulatory post-translational modifications necessary for RQC. We assessed the biological role of this arsenite-induced decrease of the regulatory ubiquitination on ribosomes using human cells transiently expressing a dual fluorescent translational reporting reporter. We found that acute exposure (18-24 hour) of 5 μM arsenite augmented the readthrough of poly(A)-containing stalling sequences within the fluorescent reporter by 11-15%, suggesting that arsenic disrupts ribosomal stalling. The role of ZNF598 in arsenic-elicited inhibition of ribosomal stalling was further validated by the knockdown of ZNF598 in the HCT116 cells. Our findings point to a novel mechanism underlying acute arsenic-induced proteostatic stress in human cells. Future investigation on the effect of chronic arsenic exposure to RQC is necessary to further elucidate the molecular etiology of arsenic-elicited proteinopathies.

2092 Shared Genetic Pathways between Metformin and Arsenic

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Arsenic is a common environmental exposure, including in parts of the United States where there is a high burden of diabetes. Urine arsenic has been associated with various endpoints in experimental models and genetic epidemiology studies evaluating whether arsenic could contribute to variable effectiveness of metformin. Further research in experimental models and genetic epidemiology studies evaluating whether these gene pathways contribute to differential metformin response in the presence of arsenic is warranted.
Chronic heavy metal exposure across the life course has the potential to alter the aging trajectory, contributing to cognitive decline and daily function. Research has established a robust relationship between environmental lead exposure and cognitive function, as measured by performance on neurobehavioral assessments. However, research suggests that instrumental activities of daily living (IADL) may be used as an alternative to measure the effect of cognitive deficits on daily routine. The present study examines the relationship between environmental lead exposure and functional status from the National Health and Nutrition Examination Survey (NHANES). Potential mediators were assessed, including impairments in auditory, visual, and motor function as well as concentration ability. Individuals with higher urinary lead levels were expected to have greater cognitive dysfunction, as indicated by greater difficulty with IADL, with lead-related impairments in auditory, visual, and motor function as well as concentration mediating this relationship. Difficulty running errands alone was associated with higher urinary lead levels (OR = 1.124, p = 0.030) and lower creatinine levels (OR = 0.998, p = 0.046). Upon the addition of demographics and potential covariates to the linear regression model adjusting for age, gender, and education, the association of lead was no longer significant. Difficulty running errands alone was associated with falling under the “other” race/ethnicity category (OR = 7.01, p = 0.035), smoking some days (OR = 2.03, p = 0.013), and experiencing difficulty walking (OR = 7.497, p < 0.001). Data suggests that environmental lead exposure in a sample of 20,146 adults from the United States is related to other-socio-demographic factors that may result in greater difficulty with IADL, indicating greater cognitive dysfunction. Further, data suggests that lead-related motor dysfunction may mediate this relationship.

Epidemiology of lead exposure has increased since the 1970s, with potential lead contamination in the environment from historical sources of lead. Despite the general decline of lead exposure in the United States, the prevalence of lead exposure remains a public health concern. The NHANES dataset provides an opportunity to examine the impact of lead exposure on cognitive function and IADL participation across the United States. This study aims to explore the relationship between urinary lead levels and IADL participation using data from the NHANES study. The study examines the relationship between urinary lead levels and IADL participation across the United States. The data shows that higher urinary lead levels are associated with greater difficulty with IADL, with lead-related impairments in auditory, visual, and motor function as well as concentration mediating this relationship. Difficulty running errands alone was associated with higher urinary lead levels (OR = 1.124, p = 0.030) and lower creatinine levels (OR = 0.998, p = 0.046). Upon the addition of demographics and potential covariates to the linear regression model adjusting for age, gender, and education, the association of lead was no longer significant. Difficulty running errands alone was associated with falling under the “other” race/ethnicity category (OR = 7.01, p = 0.035), smoking some days (OR = 2.03, p = 0.013), and experiencing difficulty walking (OR = 7.497, p < 0.001). Data suggests that environmental lead exposure in a sample of 20,146 adults from the United States is related to other-socio-demographic factors that may result in greater difficulty with IADL, indicating greater cognitive dysfunction. Further, data suggests that lead-related motor dysfunction may mediate this relationship.

To test the influence of the leaving ligand on cell survival, we exposed three cancer cell lines to the platinum(II) compound Pt(en)CBDCA: HT-29, A549, and HEK293. The IC50 values for these cell lines were determined to be 350 ± 7.12 µM, 300 ± 16.52 µM, and 80 ± 7.43 µM, respectively. The survival response was also higher in the HT-29 cells (IC50 = 300 ± 7.75 µM) compared to the HEK293 (IC50 = 200 ± 9.45 µM) cell line. Given the differences in IC50, we conclude that leaving ligand structure influences cell survival of bifunctional platinum compounds.

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Europe ranges from 1.57/1.89 to 12.5/14.6 µg/kg bw per day in the elderly and toddlers, respectively. Oral absorption of Ni is able to elicit eczematous flare-up reactions in the skin in Ni-sensitive individuals as well as adverse reproductive and developmental outcomes. Although it has low absorption (0.7-2.5%) when ingested, a BMDL01 of 1.3 mg Ni/kg bw/day was selected through dose-response modelling as a reference point for the establishment of the TDI (13 µg/kg bw). In EU legislation, there are no maximum Ni levels as a contaminant in foodstuffs, but grains and grain-based products have been identified as the most important contributor to the mean LB (lower bound) chronic dietary Ni exposure in Europe; this is despite the fact that drinking water is the only dietary source where Ni levels are regulated and have been found to be Ni-sensitized. Objective: To determine the Ni content in corn grains consumed in the Atlantic archipelagos of Cape Verde (Non-EU) and Canary Islands (EU) to characterize the risks following oral ingestion. Inductively coupled plasma optical emission spectroscopy (ICP-OES) was used to analyze 90 corn grain samples. Ni mean contents were 0.26±0.40 and 0.15±0.08 mg/kg for the Canary Islands (EU) and Cape Verde (Non-EU), respectively. Differences were observed based on various factors, with the geographic origin of the corn being the most important. A 100 g/day portion would provide 26 and 15 µg Ni, implying 3.3% and 1.9% of the TDI for the Canary Islands and Cape Verde for a 60 kg bw consumer, respectively. This confirms that cereals are a food group contributing to the total Ni dietary intake. This study contributes with information on the potential presence of Ni in a food group other than drinking water. Total diet Ni exposure assessments should be promoted across countries and age groups, and Risk Managers should develop maximum limits for those food sources of Ni in order to protect consumer health, especially in Ni-sensitized individuals where TDI may not be sufficiently protective.

2098 Methymercury Alters Carbohydrate Metabolism in Caenorhabditis elegans: Implications for Glucose Homeostasis

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Methymercury (MeHg) is a well-known neurotoxicant; however, its role in metabolic diseases has been gaining wider attention. Chronic exposure to MeHg, as measured by hair and toenail Hg levels, shows an association with diabetes mellitus (DM) and metabolic syndrome (MS). As incidences of both MS and DM are increasing globally, it is important to understand the potential role of MeHg in the development of the disease. The use of the model organism Caenorhabditis elegans can aid in identifying gene-environment interactions between MeHg exposure and MS. We have previously shown that acute exposure to MeHg alters genes involved in both lipid and carbohydrate metabolism, and causes lipid dysregulation in C. elegans. Herein we hypothesized that acute exposure to MeHg also causes carbohydrate dysregulation in nematodes. MeHg decreased total carbohydrates in worms immediately following exposure, 24 and 48 h post exposure. This loss was due to decreases in glycogen and trehalose, two storage forms of glucose in worms. Glucose levels in the worm were not significantly changed at these time points. Gene expression analysis found that carbohydrate catabolic genes pfk-1.1 (phosphofructokinase), tre-2 (trehalase), and pgpy-1 (glycogen phosphorylase) were significantly increased in response to MeHg, accounting for the decreases in glycogen and trehalose. Interestingly, gys-1 (glycogen synthase) and gys-1 (glycogenin) initially were unchanged by MeHg, but following 24 h, were significantly increased, suggesting a compensatory up-regulation in glycogen synthase to counter the loss of glycogen. Taken together, our data demonstrate that MeHg induces changes in glucose homeostasis of C. elegans that can lead to metabolic dysfunction.

2099 Role of Non-leaving Ligands in Cell Type-Specific Toxicity of Platinum(II) Compounds

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In the U.S., there will be 1,806,590 new cases of cancer this year. Among these cancer patients, half will be treated with a platinum drug during their cancer treatment. There are three FDA approved platinum drugs: carboplatin, cisplatin, and oxaliplatin, which display varying efficacy based on cell type specificity from the tissue of origin. Each drug is composed of a central platinum atom and is attached to two types of ligands: the leaving and the non-leaving. We hypothesized that the platinum drugs’ structures at the non-leaving ligands impact the cell survival in a cell-type dependent manner. To explore the impact of ligand structure on survival, we chose different non-leaving ligands in vivo. We used 4T1 breast cancer cells, which will be used in conjunction with flow cytometry to assess the extent of breast cancer metastasis to the bone follow tungsten exposure in vitro. We have previously shown that oral tungsten exposure can significantly enhance breast cancer metastasis to the lung niche, using the 66Cl4 orthotopic breast cancer model. Importantly, tungsten accumulates in the bone making it a site of long-term storage and toxicity. The bone is also a known site of breast cancer metastasis. This study was initiated to investigate the role of tungsten on breast cancer metastasis to the bone niche, using the 4T1 orthotopic breast cancer model. Similar to what was found in the 66Cl4 model, in the 4T1 model, oral tungsten (15 ppm) exposure did not affect primary tumor growth. In addition, tungsten burden in the bone was increased in 4T1 tumor-bearing mice compared to non-tumor-bearing mice exposed to 15 ppm tungsten. On day 18, micro-CT analysis performed on femur bones harvested from 4T1 tumor-bearing mice, showed a marked increase in bone osteolysis in the tungsten treated mice, including decreases in trabecular bone volume (Tb. N), bone volume/total volume ratio (BV/TV), bone surface density (BS/TV), and increases in trabecular bone separation ( Tb. Sp). Bone osteolysis is an important hallmark of metastasis to the bone niche where bone remodeling processes are disrupted leading to bone degradation. Current investigations are focused on utilization of GFP-tagged 4T1 breast cancer cells, which will be used in conjunction with flow cytometry to assess the extent of breast cancer metastasis to the bone follow tungsten exposure in vivo. In addition, quantification of bone remodeling cells (osteoblasts and osteoclasts) and serum dianimocyclohexane or ethylenediamine respectively. The MTT (3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to evaluate cell survival and determine IC50 values for each compound in multiple cellular models of human cancer and a noncancerous line, human embryonic kidney (HEK293) cells. With oxaliplatin, the IC50 for small cell lung carcinoma (A549) cells was 30 ± 6 μM, for HES293 was 43 ± 11 μM, for embryonic testicular cancer (NTERA-2) cells was 50 ± 11 μM, and for breast cancer (MCF-7) cells was 75 ± 11 μM. With Pt(en)(ox), the IC50 for melanoma (SK-MEL-5) was estimated at 40 ± 6 μM, and for the HEK293 cells was 160 ± 5 μM. From this data, we conclude that the differences in platinum compound structure at the non-leaving ligand impact survival in cell-type dependent manner.

2100 Biological Activity of an Ionic Diphenyltin Complex Synthesized from Triphenyltin Chloride


Triorganotins are popular among chemists due to their potential biological properties against bacteria, fungi, and cancer cell lines. However, they have limited applications due to their poor water solubility. We hypothesized that solubility can be improved when the complex exhibits partial ionic characteristics. Therefore, it is our research goal to synthesize triphenyltin complexes that possess ionic characteristics. Syntheses of the ionic triphenyltin complex involved the reaction of a diprotic carboxylic acid chloride with triphenyltin chloride in the presence of di-isopropylamine. However, a tin-carbon cleavage reaction was observed, as IR/NMR spectroscopy and X-ray Crystallography studies showed that an ionic diphenyltin complex was successfully obtained in the reaction. The ionic complex consists of a diphenyltin anionic moiety, two oxalate ligands, and is coordinated to the tin atom in the bidentate mode, and two disopropylammonium cations on the periphery. The anionic diphenyltin moieties has a distorted octahedral geometry with two carbons and two oxygen atoms occupying the equatorial positions and two oxygen atoms occupying the axial positions. The ionic complex is essentially a 1-D polymer and is an extensive hydrogen-bonding network of oxalate anion groups (OCO) and the N atom from the ammonium cations. Compared to the similar reaction with triphenyltin hydroxide, which resulted in ionic triphenyltin complexes, cleavage of one phenyl group was observed for the first time in the reaction with triphenyltin chloride. Due to the COVID-19 pandemic, data collection for the toxicity studies is delayed. Future studies will be focused on the mechanistic studies on the Sn-C cleavage.
2102 Micronutrients Promoting Inorganic Arsenic (iAs) Methyltion Efficiency Modify the Negative Association between iAs Exposure and Lower Birth Weight

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Inorganic arsenic (iAs) is a confirmed carcinogen and potent developmental toxicant. Prenatal exposure to iAs is associated with lower birth weight and the dysregulation of biological pathways underlying fetal growth. As part of its metabolism, iAs is sequentially methylated to mono- and di-methyl arsenicals (MMAs and DMAs). Micronutrients in the one-carbon metabolism (OMC) pathway promote the synthesis of S-adenosylmethionine, the primary methyl donor to iAs. Accordingly, optimal OMC-related micronutrient (e.g., folate and B12) levels promote methylation to DMAs and improve urinary excretion of iAs and methylated metabolites in adult populations. However, the impact of OMC-related micronutrient levels on iAs methylation efficiency during pregnancy and resulting impacts on fetal growth are understudied. Using data from a cross-sectional study of mother-infant pairs (N = 200) residing in Durango, Mexico between 2011 and 2012, we evaluated effect modification of the negative association between iAs exposure and birth weight by OMC-related micronutrients. Exposure to iAs was assessed using maternal urine and infant cord blood samples collected around the time of delivery. Serum folate, B12, and homocysteine levels were measured in maternal serum samples collected at birth. The prevalence of folate and B12 deficiencies were <2% and 74%, respectively. In comparing full (interaction + main effects) and reduced (main effects) models, we observed effect modification by B12 on the additive scale for two associations: (1) cord serum %iAs and birth weight and (2) cord serum %DMAs and birth weight. Across most analyses, we observed a consistent trend where the association between %arsenicals and birth weight was modified at lower folate and higher B12 concentrations. B12 supplementation as an intervention for iAs-associated lower birth weight.

2103 Spectroscopic and Spectrometric Approaches for Assessing the Composition of Embedded Metals in Tissues

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Many medical devices and retained material from military wounds contain metals that interface with the body. Accurately measuring the concentration of ionic metals in biological samples is essential for informing our understanding of biosolubility and distribution of solubilized metal. Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM-EDS) and X-ray Photoelectron Spectroscopy (XPS) are trusted techniques to detect and map metal fragments in tissue but may not have the sensitivity to detect low concentrations of metal ions in the peri-implant tissue. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is a potential alternative that preserves spatial information while being capable of trace elemental analysis. To this end, single-element metal pellets of Al, Co, Cu, Fe, Ni, Pb, Ta, or W were implanted into the gastrocnemius muscle in rats for up to one year, the peri-implant tissue flash frozen, then cryosectioned for histology and mapping of metal distribution using SEM-EDS, XPS, and LA-ICP-MS. Histological analysis revealed inflammation and tumor formation around the implantation site of rats with Ni pellets. LA-ICP-MS identified the elemental composition of a Pb fragment but ineffective for trace metal detection; samples containing dissolved metal must be approached differently than those containing retained metal pieces. XPS was unable to detect metal in the tissue samples. As an alternative, we used LA-ICP-MS to preserve spatial information and perform trace elemental analysis, showing hundreds of parts-per-million concentrations of metal in some tissues. LA-ICP-MS is appropriate for the detection of trace ionic metal in tissue samples of this type, although it is not the obvious first choice. The long-term health consequences of many of these materials are not thoroughly understood, but detecting them reliably is a necessary step toward their investigation. Additionally, the ability to correlate histological features with metal ion concentration in tissue is essential to elucidating the local effects of implanted/retained metals on tissue.
Chronic exposure to inorganic arsenic (iAs) has been linked to diabetic phenotypes in humans and mice. However, the diabetogenic effects of iAs exposure during specific developmental windows have never been systematically studied. In mice, we have previously shown that combined preconception and in utero exposures to iAs resulted in impaired glucose homeostasis in male offspring. The current study aims to determine if preconception exposure alone can contribute to this outcome. We hypothesized that preconception exposure to iAs would lead to changes in transcript levels in parental germ cells and result in an adverse metabolic phenotype in offspring. We further hypothesized that transcription profiles in target tissues from offspring would reflect the preconception-associated germine transcription. We examined metabolic phenotypes in male and female offspring from dams and sires exposed to iAs in drinking water (0 or 200 µg As/L) for ten weeks prior to mating. The effects of iAs exposure on gene expression profiles in parental germ cells, and pancreatic islets and livers from offspring were assessed using RNA sequencing. We found that iAs exposure significantly altered transcript levels of genes, including diabetes-related genes, in the sperm of sires. Notably, some of the same genes were also shared between the same parents exposed to iAs and those exposed to a control diet. This suggests that preconception exposure may confer a risk for diabetes in offspring. In summary, our study highlights novel findings suggesting that preconception iAs exposure plays a role in glucose dysregulation in adult mice in a sex-specific manner.
**2110** Liver Toxicity Observed with Lorlatinib When Combined with Strong CYP3A Inducers: Evaluation of Cynomolgus Monkey as a Nonclinical Model for Assessing the Mechanism of Combinational Toxicity


Lorlatinib is a potent small molecule anaplastic lymphoma kinase (ALK) inhibitor approved for the treatment of patients with non-small cell lung cancer. In a drug-drug-interaction (DDI) study in healthy human participants, liver enzyme elevations were observed when a single 100 mg dose of lorlatinib was administered after multiple doses of rifampin, a strong cypochrome P450 (CYP) 3A inducer and a pregnancy X receptor (PXR) agonist. A series of in vitro and in vivo studies were conducted to evaluate potential mechanisms for the observed clinical toxicity. To investigate the involvement of CYP3A and/or PXR in the observed liver toxicity, studies were conducted in cynomolgus monkeys administered lorlatinib alone or with co-administration of multiple doses of known CYP3A inducers that are predominantly PXR agonists (rifampin, St. Johns wort) or predominantly constitutive androstane receptor (CAR) agonists (carbamazepine, phenytoin) and a net CYP3A inhibitory PXR agonist (rimonavir). Results from the investigative studies identified cynomolgus monkeys as a pharmacologically relevant nonclinical model, which recapitulated the elevated liver function test results observed in humans. Furthermore, liver toxicity was only observed in this model when lorlatinib was co-administered with strong CYP3A inducers and the effects were not restricted to, or exclusively dependent upon, a PXR activation mechanism.

**2111** In Vitro Metabolism of Naked versus Alkylated Polycyclic Aromatic Hydrocarbons (PAHs) That May Be Present in the Mineral Oils

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Hydrocarbons may be found in foodstuffs. Although some of the hydrocarbons in food are of natural origin, mineral oil residues originating from food packaging, additives, processing aids, and lubricants may also be present. An emerging concern in modern food safety is the possible carcinogenicity of aromatic hydrocarbons that may be present in mineral oil and end up in foodstuffs. The bioactivation of non-substituted PAHs has been extensively studied in relation to carcinogenicity. However very few data on possible metabolic activation of alkyl substituted PAHs, which are predominantly present in mineral oils, have been reported. The present study investigates the oxidative metabolism of PAHs and their alkylated congeners by rat and human rat liver microsomes to get a better insight in the relative balance between bioactivation and detoxification. Model compounds included in the study were naphthalene, phenanthrene, benzo[a]pyrene and some of their methyl-, ethyl-, n-hexyl-, and n-dodecyl-substituted analogues. Metabolite formation was evaluated using gas chromatography-mass spectrometry (GC-MS). Five of the alkylated PAHs were selected for a detailed investigation. The formation of metabolites followed a sequence of elongation of the alkyl side chain, in favor of detoxification and excretion. Furthermore, elongation of the alkyl side chain reduced the overall metabolism of PAHs. In summary, alkylation of PAHs may increase the chances of alkyl chain oxidation and detoxification and as a consequence reduce the genotoxic risk.

**2112** Development of a Strategy to Determine the In Vitro Induction of T4-Glucuronidation in Rat and Human


The thyroid hormones (THs) triiodothyronine (T3) and thyroxine (T4) are essential for normal cellular differentiation. The prohormone T3 can be converted enzymatically into the biologically active T3, and as such functions as a reservoir for T3. Xenobiotics can alter the homeostasis of THs through various different pathways. One of the pathways by which xenobiotics may cause effects on TH homeostasis is by induction of Phase I and Phase II enzymes, especially hepatic glucuronidation, resulting in increased metabolism. Both T3 and T4 can be glucuronidated and the formed glucuronides can no longer be converted into active T3 and are biliary excreted. As there are notable differences in the systemic regulation of THs between experimental animal models and humans, it is important to determine if specific thyroid effects observed in animal models are human relevant and this includes induction of hepatic glucuronidation. Therefore, a strategy to determine induction of T4-glucuronidation in different species (rat and human) was evaluated. The strategy combined the use of gene expression (mRNA analysis) and enzyme activity as endpoints for induction of T4-glucuronidation and included cytochrome P450 (CYP) 3A4 inducer and a pregnancy X receptor (PXR) agonist. A series of in vitro and in vivo studies were conducted to evaluate potential mechanisms for the observed clinical toxicity. To investigate the involvement of CYP3A and/or PXR in the observed liver toxicity, studies were conducted in cynomolgus monkeys administered lorlatinib alone or with co-administration of multiple doses of known CYP3A inducers that are predominantly PXR agonists (rifampin, St. Johns wort) or predominantly constitutive androstane receptor (CAR) agonists (carbamazepine, phenytoin) and a net CYP3A inhibitory PXR agonist (rimonavir). Results from the investigative studies identified cynomolgus monkeys as a pharmacologically relevant nonclinical model, which recapitulated the elevated liver function test results observed in humans. Furthermore, liver toxicity was only observed in this model when lorlatinib was co-administered with strong CYP3A inducers and the effects were not restricted to, or exclusively dependent upon, a PXR activation mechanism.

**2113** CYP Machine-Learning Models for Predicting Metabolism and Drug-Drug Interactions of Xenobiotics


Little is known about the human metabolism of organophosphate (OP) pesticides. Accidental exposure and intentional poisoning by OP pesticides (OP) kills hundreds of thousands of people every year. Over the past 20 years, large quantities of in vitro and in vivo data have accumulated on drug metabolism in humans. Machine learning methods have been applied to many datasets toxicological research to enable prospective prediction and increase efficiency by minimizing testing. The availability of drug metabolism and inhibition data enables the building of predictive computational models using molecular structure. We have curated data for human drug metabolizing enzymes from CHEMBL, PubChem and other sources, followed by building of Bayesian and other machine learning models with our Assay Central software. Individual CYP K\textsubscript{m} data are remarkably limited such that manual extraction and curation of these data from the public domain are necessary in order to then generate these machine learning models. There are publicly accessible data for drugs (CypReact, drugbank.ca, bioinformatics.charite.de, etc) as substrates for CYPs identified through various experimental techniques. Using these data for approximately 1600 compounds, we have developed Bayesian models for all the major CYP isozymes and with the inclusion of 1000 dummy (XenoSite), negative compounds produced cross-validation ROCs of 0.892, 0.851, 0.867, 0.875, 0.902, 0.926, for CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6 and CYP3Aorefore. As much of this data is qualitative, these data are binary. Through manual curation we have also compiled K\textsubscript{m} and V\textsubscript{max} data for hundreds of additional compounds, which we have also modeled with variable success. These models were then used to predict 23 OP compounds followed by in vitro verification. The results showed poor predictive ability for OP metabolism, likely due to the lack of representative organophosphate compounds in the training sets. Independently, we have also assessed the clearance rates for five of these compounds in microsomes and have compared these results to those predicted using a relative activity factor (RAF) model. We have also used this test set to create an independent model as well as adding these data to the larger CYP models to improving their predictive capabilities.

**2114** Identifying Arachidonic Acid as a Candidate Orphan Substrate of CYP1B1 in the Eye Using Untargeted Metabolomics and an Improved Capillary Morphogenesis Assay


Little is known about the human metabolism of organophosphate (OP) pesticides. Accidental exposure and intentional poisoning by OP pesticides (OP) kills hundreds of thousands of people every year. Over the past 20 years, large quantities of in vitro and in vivo data have accumulated on drug metabolism in humans. Machine learning methods have been applied to many datasets toxicological research to enable prospective prediction and increase efficiency by minimizing testing. The availability of drug metabolism and inhibition data enables the building of predictive computational models using molecular structure. We have curated data for human drug metabolizing enzymes from CHEMBL, PubChem and other sources, followed by building of Bayesian and other machine learning models with our Assay Central software. Individual CYP K\textsubscript{m} data are remarkably limited such that manual extraction and curation of these data from the public domain are necessary in order to then generate these machine learning models. There are publicly accessible data for drugs (CypReact, drugbank.ca, bioinformatics.charite.de, etc) as substrates for CYPs identified through various experimental techniques. Using these data for approximately 1600 compounds, we have developed Bayesian models for all the major CYP isozymes and with the inclusion of 1000 dummy (XenoSite), negative compounds produced cross-validation ROCs of 0.892, 0.851, 0.867, 0.875, 0.902, 0.926, for CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 respectively. As much of this data is qualitative, these data are binary. Through manual curation we have also compiled K\textsubscript{m} and V\textsubscript{max} data for hundreds of additional compounds, which we have also modeled with variable success. These models were then used to predict 23 OP compounds followed by in vitro verification. The results showed poor predictive ability for OP metabolism, likely due to the lack of representative organophosphate compounds in the training sets. Independently, we have also assessed the clearance rates for five of these compounds in microsomes and have compared these results to those predicted using a relative activity factor (RAF) model. We have also used this test set to create an independent model as well as adding these data to the larger CYP models to improving their predictive capabilities.
wound repair, but a common mechanism or signaling agent remains unknown. To help identify new potential endogenous substrates for CYP1B1 in the eye, we performed untargeted metabolomics on conditioned media from retinal endothelial cells (REC) taken from both wild-type (WT) and CYP1B1 (-/-) KO mice. WT REC cells form normal capillaries when grown on an extracellular matrix such as Matrigel, but CYP1B1-KO REC cells do not. We hypothesized that cell lysates from CYP1B1-KO REC cells might help with the formation of microvessels by accumulating potential substrates (and lack the CYP1B1 metabolite) required to promote normal capillary morphogenesis. Untargeted metabolomics comparisons of conditioned media from WT and CYP1B1-KO cells, identified 295 chemicals differentially present. After heat map analysis we binned the top 20 hits (in terms of metabolite enrichment in CYP1B1-KO REC cells) into 10 categories, and the top 20 hits were prioritized for screening in a 96-well capillary morphogenesis assay that measures changes in capillary branch formation rates between treated cell populations. The 96-well assay was validated by demonstrating that CYP1B1-KO REC cell morphogenesis can be rescued with conditioned media from WT REC cells expressing CYP1B1, and CYP1B1’s discrete role in this process was verified using the selective CYP1B1 inhibitor, (Trans-2,3,4,5-tetramethoxystilbene) TMS. Capillary branching was reduced over 90% in WT REC cells treated with TMS (1-100 µM; IC₅₀ = 4.91 µM). Two compounds identified by untargeted metabolomics (arachidonic acid and hypoxanthine) were verified as potential endogenous substrates for CYP1B1 using the capillary morphogenesis assay. Arachidonic acid was most notable, as it was shown to significantly stimulate capillary branching in CYP1B1-WT cells at low nanomolar levels (30 nM), and this stimulatory effect could be suppressed with co-administration of TMS (50 µM). While CYP1B1’s potential role in arachidonic acid metabolism had been previously described, this study provides independent supporting evidence that arachidonic acid is an endogenous substrate for CYP1B1 in the mammalian retina. While the metabolic fate of arachidonic acid in REC cells remains unknown, we speculate that CYP1B1 may be functioning as a CYP epoxygenase in the eye, capable of generating epoxygenoarachidonic acids (EETs) like 5,6-EET, which are known regulators of endothelial cell growth and differentiation processes in cancer.

**2115 Metabolism of Naphthalene by Mouse Liver and Lung Microsomes: Glutathione Does Not Affect 1,2-Dihydrodiol Formation**

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Naphthalene (NA) is a ubiquitous carcinogenic pollutant to which humans are widely exposed. A prerequisite for NA’s toxicity in the respiratory tract is bioactivation by cytochrome P450 (CYP) enzymes. NA metabolic pathways to toxicity involve initial formation of NA-oxide, and subsequent formation of re-active metabolites derived from 1,2-NA-dihydrodiol (producing 1,2-naphthaquinone) or 1-naphthol (producing 1,4-naphthoquinone). However, the relative importance of these reactive metabolites and more specifically the role of reduced glutathione (GSH), which reacts with NA-oxide to form NA-GSH adducts, in modulating the abundance of 1,2-NA-dihydrodiol and 1-naphthol is unclear. In this study, we examined the effects of GSH and UDPGA (cofactor for UDP-glucuronoyl transferase) addition on the rates of 1,2-NA-dihydrodiol and 1-naphthol formation in reactions of NA with mouse liver microsomes. Levels of 1,2-NA-dihydrodiol and 1-naphthol were measured using UPLC-UV, whereas those of NA-GSH were measured using LC-MS/MS. We show that addition of GSH up to 10 mM, which led to maximal formation of NA-GSH, did not significantly decrease rates of 1-naphthol or 1,2-NA-dihydrodiol formation in vitro. On the contrary, UDPGA addition resulted in significant decreases in 1-naphthol formation, but not 1,2-NA-dihydrodiol formation. These results suggest that GSH cannot prevent formation of 1,2-NA-dihydrodiol, a reaction catalyzed by microsomal epoxide hydrolase. Similarly, in lung microsomal incubations, addition of GSH did not decrease rates of 1-naphthol or 1,2-NA-dihydrodiol formation. The implication from these findings is that levels of NA-GSH may not correlate with those of 1-naphthol or 1,2-NA-dihydrodiol formation in vivo. In NA-exposed individuals, and that the risks of toxicity from the three different bioactivation pathways (via the formation of NA-oxide, 1,2-NA-quinone, and 1,4-NA-quinone) should be assessed independently. Supported in part by NIH grant ES020867.

**2116 The Dichloroacetamide Safener Benoxacor Is Enantioselectively Metabolized in Monkey Liver Microsomes and Cytosol**

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Safeners like benoxacor are inert ingredients of herbicide formulations; however, only little information is available about their metabolism and toxicity. The goal of this study was to elucidate the enantioselective metabolism of benoxacor in vitro. We studied the depletion of benoxacor in microsomes (5 µM benoxacor, 0.1 mg/mL microsomal protein, 1 mM NADPH, 37°C) and cytosol (5 µM benoxacor, 1 mg/mL protein, 5 mM glutathione, 37°C) prepared from female and male monkey livers. Benoxacor levels were determined by gas chromatography-mass spectrometry. Enantiomeric fractions (EF) were determined by enantioselective gas chromatography with an electron capture detection method. Benoxacor and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were added to monkey liver microsomes and cytosol. After 30 minutes, 18±1% and 26±3% of benoxacor remained in incubations with microsomes, and 29±2% and 32±1% remained in incubations with cytosol obtained from female and male monkeys, respectively. Without the addition of NADPH in the microsomes, 68±1% and 63±1% of benoxacor remained in microsomes obtained from female and male monkeys, respectively. The value returned to near racemic and then the first eluting enantiomer of benoxacor (E₁-benoxacor) was enriched for longer incubation times. This is likely due to a different direction of the enantioselective metabolism of benoxacor by cytochrome P450s and carboxylesterases. A more pronounced enrichment was noted at 30-minutes in incubations with microsomes from female than male monkeys (EF = 0.66±0.01 vs. EF = 0.54±0.01, respectively). Incubations with cytosol from both female and male monkeys resulted in the complete depletion of E₁-benoxacor, while levels of E₁-benoxacor remained relatively unchanged. These results indicate that microsomal and cytosolic enzymes from monkeys enantioselectively metabolize benoxacor. Further research is needed to determine the effects of enantioselective metabolism on the toxicity of benoxacor.

**2117 Development and Validation of Two CRISPR/Cas9-Generated Cytochrome P4501A1 (CYP1A1) Zebrafish Lines**

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Cytochrome P450s (CYPs) are a superfamily of enzymes responsible for phase one metabolism of a broad range of endogenous and exogenous ligands. CYP1A is highly induced by exposure to aryl hydrocarbon receptor ligands, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and some polycyclic aromatic hydrocarbons (PAHs). CYP1A has been implicated in both detoxification and metabolic activation of ligands in zebrafish, but the lack of available stable CYP1A mutants has hindered our ability to fully understand CYP1A’s role in specific AhR ligand toxicity. To investigate the role of CYP1A in xenobiotic metabolism and toxicity, two mutant lines were generated using CRISPR/Cas9: a 2 base pair (CYP1A-2bp) deletion and a 15 bp (CYP1A+15bp) insertion in exon 2 of the cyp1a gene. The CYP1A-2 bp line results in a premature stop codon leading to a truncated protein at amino acid 14 in the 519 amino acid full length protein. The CYP1A+15bp line is an in-frame insertion of five amino acids at amino acid 121. To investigate the role of the CYP1A enzyme in these lines, localization of the CYP protein using immunohistochemistry (IHC) and catalytic activity using the ethoxyresorufin-o-deethylase (EROD) assay were performed in larval mutants. Whole mount IHC and in vivo EROD assays both showed reduced CYP1A protein expression and activity in the CYP1A+15bp line. CYP1A protein expression and EROD activity were completely absent in the CYP1A-2bp line, indicating successful knockout of CYP1A in this line. Additionally, fish from each mutant line were exposed to environmentally and toxicologically relevant AhR ligands followed by the evaluation of 13 morphological and two behavioral endpoints. Exposure of each line to TCDD resulted in no apparent differences in TCDD bioactivity relative to wild type fish, which would be expected of a compound not metabolized by CYP1A. Exposure of the two mutant lines to retene, an AhR ligand known to undergo phase one metabolism, resulted in significantly increased bioactivity. Retene bioactivation which induced 50% effects (EC₅₀) upon retene exposure shifted from 47 µM in wild type fish to 7.99 and 9.71 µM in the CYP1A-2bp and CYP1A+15bp lines, respectively. Each evaluation supports the successful development of CYP1A-deficient zebrafish lines which will serve as valuable tools to assess the in vivo role of CYP1A-dependent metabolism on xenobiotic responses.
Phase I cytochrome P450s commence the metabolism of polycyclic aromatic hydrocarbons (PAHs), catalyzing chemical reactions to enhance solubility, while Phase II enzymes conjugate polar metabolites to further enhance solubility and elimination. Bioactivation of PAHs can also occur, resulting in metabolites with increased toxicity over the parent compound. While it is important to identify key enzymes involved in detoxifying or bioactivating PAHs, methods for global identification of enzymes involved in PAH metabolism are lacking. We developed a high-throughput method using activity-based proteomics to identify cytochrome P450s involved in PAH metabolism using the model PAH, benzo[a]pyrene (BaP). Our objective herein is to identify specific P450 enzymes active in the metabolism of BaP in human hepatic microsomes at multiple substrate concentrations. We co-incubated BaP at low and high concentrations (6.25 and 20 µM) with the activity-based probe 2-ethylencylnaphthalene (2-EN), specific for P450s, in pooled human liver microsomes (pool of 200 individuals) using a kinetic metabolism assay developed in our laboratory. We enriched probe-labeled, active P450 enzymes and measured proteins using liquid chromatography-mass spectrometry. When BaP was co-incubated with 2-EN, we observed a decrease in enriched enzymes compared to probe-only controls, allowing us to identify specific enzymes involved in BaP metabolism. Preliminary data suggest CYPs 1a2, 2c19, 2d7, 4f2, and 5a1 to be the key enzymes active in BaP metabolism in human liver microsomes. A kinetics assay of BaP (5 µM) co-incubated with 2-EN (10 µM) revealed no inhibition of BaP metabolism by the probe in human liver microsomes at the concentrations tested. We are currently evaluating other substrate concentrations to identify important enzymes throughout the Michaelis-Menten curve and repeating these experiments to investigate P450s active against two additional PAHs, retene and phenanthrene. This novel approach is a powerful tool that can be used to identify P450 enzymes involved in human metabolism of PAHs as well as probe additional Phase II enzyme families of interest. The ability to identify specific enzymes active in PAH metabolism will greatly aid our efforts to determine health risks of PAH exposures to individuals.

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**2122 The HRTPT Cells as a Model System of Progenitor/Stem Cells Involved in Renal Tubular Regeneration after a Toxic Insult**

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The renal tubules function in reabsorption after filtration in each nephron unit of the kidney and are target sites of injury due to exposure to toxicants. Damage to the tubules can lead to conditions such as acute kidney injury, chronic kidney disease, and ultimately end-stage renal failure (ESRF). Renal tubules have the ability to regenerate after acute injury suggesting the presence of stem/progenitor cells in the kidney. In our previous study, we utilized an immortalized human kidney proximal tubular epithelial cell line (HRTPT) and the HREC24T. The HRTPT cells co-express the stem cell markers CD133 and CD24, whereas the HREC24T cells only express CD24. Further characterization of these cells showed that the HRTPT cells formed nephrospores, and had the ability to grow and undergo adipogenic, neurogenic, tubulogenic and osteogenic differentiation, showing most of the key characteristic features of stem cells. The HREC24T cells were unable to form spheres and failed to undergo differentiation, suggesting that they lacked stem/progenitor cell features.

In this study, global gene expression analysis was performed on the HRTPT and HREC24T cell lines using the Affymetrix Microarray platform. The analysis identified 873 genes that were specific for HRTPT cells. Further analysis using the DAVID and Reactome Pathway Knowledge Base identified 35 genes that were significantly upregulated in the HRTPT cells. The expression of these genes was validated by droplet digital PCR. Most of the genes identified either were components of the cytoskeleton, or were involved in the regulation of the cytoskeletal structures particularly the microtubules. In conclusion, our data suggests that the HRTPT cells express a unique set of genes and can serve as a model system to determine the role of progenitor/stem cells in renal repair and regeneration after a toxic insult.

**2123 Desorption Electrospray Ionization Mass Spectrometry Imaging (DESI-MSI) of Acetaminophen and its Metabolites in the Kidney**

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Acetaminophen (APAP) overdose is the most common cause of acute liver failure in the US and a significant number of patients develop kidney injury, which can progress to renal failure. Mechanisms of APAP-induced nephrotoxicity have received comparatively less attention than those involved in liver injury, though the main cause of APAP-induced kidney injury is presumed to be APAP bioactivation and glutathione depletion in renal tubular cells of the kidney cortex. While biomarkers of APAP metabolism and injury are typically assessed in whole tissue homogenates, this lacks the spatial information relevant to understanding mechanisms of APAP-induced nephrotoxicity. Desorption Electro-Spray Ionization Mass Spectrometry Imaging (DESI-MSI) allows the simultaneous generation of ion images for APAP and its metabolites under ambient air, without chemical labeling or prior coating of tissue which may induce chemical interference or perturbation of small molecule localization. To gain spatially relevant information on APAP metabolism in the kidney and its relationship with various renal compartments, male C57Bl/6J mice were treated with 300, 600, 900 or 1200 mg/kg APAP for 30 minutes, followed by evaluation of drug metabolism in the kidney, liver and plasma. Whole kidney tissue treated with APAP treatment resulted in almost complete GSH depletion in the liver, this was less pronounced in the kidney. Measurements of APAP, its metabolites and APAP-protein adducts revealed a dose dependent increase of these parameters in the whole kidney like the liver. Notably, DESI-MSI visualization of the spatial intensity and tissue distribution of metabolites from histological sections provided identification and spatial localization of APAP, APAP-glucuronide and endogenous lipids which were the most abundant compounds in the kidney inner medulla after APAP treatment. This topographical distribution was confirmed by laser capture microdissection of kidney sub-compartments and metabolite analysis by MS. In conclusion, our results indicate that DESI-MSI provides an innovative platform to characterize spatial alterations in APAP metabolite abundance after an overdose in the kidney and could be used to investigate the influence of therapeutic interventions on these parameters to provide mechanistic insight into their prevention of APAP-induced nephrotoxicity.

**2124 In Vitro Modeling of Synergistic Effect(s) of Heat Stress and Ochratoxin A as Risk Factors for CKDu**


Chronic Kidney Disease of Unknown Etiology (CKDu) is an area of ongoing human health concern but, thus far, definitive causal factors from epidemiological studies have been equivocal. Ochratoxin A (OTA), a fungal mycotoxin originating from Penicillium and Aspergillus species, is often found in food products like grains, coffee, and fruit. OTA is hypothesized to be a contributing factor to the development of CKDu because it is present in the blood and urine of individuals with CKDu. Epidemiological studies have found CKDu to be common among residents of warm climates like Sri Lanka, Indonesia, and Central America. To investigate the hypothesis that there is a synergistic effect between OTA exposure and transient hyperthermia, primary human proximal tubule epithelial cells (PTECs) were cultured in the presence of OTA (0-10µM) and heat stress (24 hr. at 39°C). The effect of heat and OTA on PTECs was analyzed using RNA sequencing to examine global changes in gene expression in order to identify mechanism(s) of OTA toxicity and delineate the effect of heat and/or OTA on PTECs. Preliminary RNA sequencing data was analyzed for an increase in the expression of CDKN1A, which codes for p21, an inhibitor of cell cycle regulating cyclin dependent kinases (CDK1, CDK2, CDK4/6). Increased expression in p21 can lead to arrest of the cell cycle in G1/S and G2/M phase, which has been identified as a possible cause of renal injury leading to CKD. This increase in CDKN1A expression was absent in cells treated with 0 µM OTA +/- heat. Most importantly, there was an increase in CDKN1A expression in cells that were treated with OTA and a greater increase in expression was seen in cells that were treated with OTA exposed to transient hyperthermia (P <0.001), providing evidence that heat and OTA have a synergistic, nephropathic effect on PTECs. This demonstrates that environmental and dietary factors should be considered in assessing risk of developing CKDu and lays the groundwork for delineating the complicated mechanisms of this disease. Ongoing studies are assessing the gene X environment interactions of GSTs in OTA nephrotoxicity utilizing our 3D microphysiological model of the human kidney.

**2125 Downregulation of Lysosomal and mTOR-Related Genes in Human Renal Tubular Epithelial Cells Composed of the Progenitor CD133+/CD24- Cells and CD24+ Cells by Elevated Glucose**

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Hyperglycemia is one of the major health concern in many parts of the world. One of the serious complications of high glucose levels is diabetic nephropathy. The preliminary micro-array study performed on primary human renal tubular epithelial (hRTE) cells exposed to high glucose levels showed a significant down-regulation of mTOR as well as its associated genes as well as lysosomal genes. Based on this preliminary data, the expression of various lysosomal genes as well as mTOR and its associated genes were analyzed in hRTE cells exposed to 5.5, 7.5, 11 and 16 mM glucose. The results validated the microarray analysis, which showed a significant decrease in the mRNA as well as protein expression of the selected genes as the concentration of glucose increased. Co-localization of lysosomal marker, LAMP1 with mTOR showed lower expression of mTOR as the glucose concentration increased, suggesting decrease in mTOR activity. Although the mechanism by which glucose affects the regulation of lysosomal genes is not well known, our results suggest that high levels of glucose may lead to decrease in mTOR expression causing the cells to enter an anabolic state with subsequent down-regulation of lysosomal genes.
In toxicology and pharmacology studies, glomerular filtration rate (GFR) is considered a gold standard in assessing renal function. The renal clearance of labeled inulin measured by photometers has been known as a filtration marker for the determination of GFR. Preclinically, a non-invasive GFR measurement method was recently developed, in which near-infrared fluorescent-labeled form of inulin is scanned with fluorescence molecular tomography (FMT). In this study, we demonstrated that an IVIS Spectrum based method for determining GFR using fluorescence-labeled inulin (GFR-Vivo 680) was evaluated in conscious male C57BL/6 mice orally administered with vehicle or immunosuppressive agent - cyclophosphamide (Csa, 80 mg/kg) for 14 days. Based on a two-compartment model fitting, estimated GFR (eGFR) was 235.40 ± 52.46 and 188.94 ± 18.97 µl/min; p<0.01 in vehicle-treated and Csa-treated mice (C57BL6/J), respectively. The results corresponded well to FMT imaging showing a reduction in GFR in the CsA treated mice, which yielded comparable GFR values (229.21 ± 60.96 and 150.80 ± 35.06 µl/min, p<0.01), together with previously reported values in literature. We propose this alternative, simple, less stressful, and versatile way to measure GFR ex vivo and in vivo using a popular IVIS system and convenient inulin probe in toxicology studies.

2126 A Novel Ex Vivo and In Vivo Hybrid IVIS Imaging of GFR-Vivo 680 Provides a Convenient and Precise Technique for Measurement of Glomerular Filtration Rate in Conscious Mice
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Tetrabromobisphenol A (TBBPA), hexabromocyclododecane (HBCD), and polybrominated diphenyl ethers (PBDEs) are three BFRs that occupy nearly 20 percent of the global flame retardant market and are reported to have adverse effects on humans and wildlife. We previously determined the effects of TBBPA, HBCD, and BDE 47 on MTT staining and cell morphology in rat (NRK 52E), adult human (HK-2) kidney cells after 48 hours of exposure, and demonstrated that these compounds induce concentration- and cell-dependent toxicity, with human cells being more susceptible. We investigated the mechanisms underlying this cell-dependent toxicity by performing RNAseq analysis. Principal components analysis supported the hypothesis that BFRs induce differential transcriptional changes in rat and human cells. Furthermore, within each cell line, only 9 differentially expressed genes and 5 differentially expressed genes were shared between all 3 BFRs in rat and human cells, respectively. This suggests that the molecular mechanisms mediating BFR toxicity are toxicant and cell line-dependent. Gene set enrichment analysis demonstrated some similarities in the functional consequences of BFRs that persisted across different cell lines. Namely, changes in extracellular matrix turnover and the coagulation cascade were shared across all 3 chemicals in both cell lines. Taken together, these data will aid future studies concerning the molecular mechanisms of BFR nephrotoxicity and risk assessment.

2129 Development of a 3D In Vitro Screening Model to Monitor Kidney Proximal Tubule Toxicity
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Drug induced kidney proximal tubule injury is a major reason for compound attrition and there is a need to develop robust in-vitro screening assays to identify nephrotoxic compounds before they reach the clinic. Newer 3D microfluidic technologies allow culture of cells under physiological stress thus better recapitulating human biology. To that end, a microfluidic model using human primary renal proximal tubule epithelial cells (hRPTECs) was developed with relevant stress biomarkers to enable better in vitro in vivo correlation. The hRPTECs were cultured in a medium-throughput microfluidic device for seven days to obtain tubules with a leak-tight barrier. Different tool compounds including cisplatin, gentamicin, carboplatin and streptomycin (non-nephrotoxic) were then added at multiple doses and cultured for 72 hrs. Cell viability was determined by WST and LDH assays, and barrier integrity were performed using FITC and TRITC-dextran. Several tubular injury biomarkers (KIM-1, clusterin and NAG) and a panel of four micro-RNAs (miR-21, miR-29a, miR-34a and miR-192) were measured in the supernatant. A dose-dependent change in all miRNAs and a strong increase of NAG concentration in medium was observed for nephrotoxic compounds over non-nephrotoxic compounds, which slightly preceded changes in barrier function and cytotoxic effects. First studies on KIM-1 and clusterin resulted in a dose-dependent increase after treatment with cisplatin. Thus, this assay shows its potential as a useful screening platform for detection of proximal tubule injury.

2130 Cisplatin Renal Cytotoxicity and Mitochondrial Alterations Are Modified by Resveratrol in Human Proximal Tubular Epithelial Cells
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The cancer chemotherapeutic agent cisplatin is part of the treatment regimen for treating advanced testicular, ovarian and bladder cancer. Cisplatin treatment increases the risk of permanent renal dysfunction in vitro and in vivo. Cisplatin nephrotoxicity targets in humans, the proximal and distal tubules. Resveratrol (RES) is a natural compound with antioxidant activity and promotes apoptosis in some cancer cells. RES has diminished tumor size in clinical studies. RES reduces cisplatin nephrotoxicity. Part of the mechanism for reducing cisplatin toxicity by RES may be mediated by preserving mitochondrial function and diminishing cisplatin induced oxidative stress. HK-2 cells which are a human renal proximal tubular epithelial cell line (HK-2) were exposed to cisplatin in the presence of RES. 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 pretreated with RES or 1% DMSO (vehicle). Renal cells were co-incubated with 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 decrease in cell viability after 24 h exposure to 0, 7.5 and 15 µM cisplatin. RES pretreatment for 1 h with 10 or 15 µM RES increased HK-2 viability relative to cells exposed only to RES
Cisplatin is a chemotherapeutic drug that is effective in treating head and neck, lung, ovarian and testicular cancers. However, the clinical utility of cisplatin is often limited by its accumulation in proximal tubule cells leading to acute kidney injury. The renal secretion of cisplatin is accomplished by the uptake organic cation transporter 2 (OCT2) on the basolateral side and the multidrug and toxic extruder 1 (MATE1) efflux transporter located on the apical side of proximal tubule cells. In the current study, we sought to develop a novel in vitro model of human OCT2- and MATE1-mediated transepithelial transport of cisplatin across a polarized monolayer. MDCK cells expressing an empty vector (EV) or double transfected with human OCT2 and MATE1 (OCT2/MATE1) genes were grown in Transwells inserted to create distinct basolateral (blood) and apical (urinary filtrate) compartments. OCT2 (1, 5, 10 µM) was applied to the basolateral side. Time-dependent secretion and accumulation of platinum in the apical compartment over 120 min was measured using ICP/MS. TEER was measured at the beginning and end of experiments to ensure monolayer integrity. OCT2/MATE1 cells exhibited concentration- and time-dependent secretion of platinum into the apical compartment that was more extensive in the OCT2/MATE1 cells. Notable differences in platinum transport were observed in the Transwells treated with 5 and 10 µM cisplatin. By 120 min, the concentration of platinum in the apical chamber of the OCT2/MATE1 cells was over 50% higher than the EV cells. The net secretion rate of platinum attributable to OCT2 and MATE1 was two-fold higher at 10µM compared to the 5 µM cisplatin treatments. Taken together, these data support use of OCT2/MATE1 cells to assess transporter-dependent transepithelial transport of cisplatin across a kidney monolayer. This model will be advantageous for evaluating novel cisplatin-drug interactions. Supported by R01GM123330, UL1TR003017, and P30ES005022.

Drug-Induced-Liver-Injury (DILI) is one of the most significant reasons why drug development or sales are discontinued. Due to the complicated mechanistic underpinning of DILI, it is still very difficult to predict the toxicity before or even after clinical study phase. Researchers on predicting such toxicity by using in vitro hepatic tissue constructed from human-derived cells have been developed in many groups. But it is also difficult to mimic highly ordered structure like blood capillaries, and no hepatic-toxicity assessment system hasn’t reached the level of constructing a hepatic vascular model, and toxicity assessment system by using the model. We constructed a liver-like tissue model consisted from human hepatocytes from xenogeneic host livers (PXB cell), Sinusoidal Endothelial Cell (SEC), and hepatic stellate cell (LX-2). In this case, we coated the surfaces of those cells with collagen and heparin for in vitro tissue construction. Collagen and heparin interact with the molecules on the cell surface, which can induce cell-cell adhesion suitable for constructing a tightly arranged cell model like in vivo liver. We have achieved both the construction of the vascularized hepatic model and a ratio of initial hepatocytes close to in vivo (65%). And the models were used to test the acute hepatotoxicity of chemicals, at least within 14 days without drastic decreasing (≤1000ng/10^6 cells/day). We confirmed the predictive performance of DILI by treating 10 training compounds selected in the Dragovic’s report [1]. Our model showed a higher predictive power for some compounds compared to traditional 2D cultures [2]. We also tried to evaluate a sinusoidal obstruction syndrome (SOS)-inducing compound monocrotaline. Since the characteristic symptom of SOS is sinusoid attenuation, we evaluated the toxicity by using capillary image analysis. It predicted the toxicity over 10 times more sensitively than the usual viability assay. [1] S. Dragovic et al., Arch. Toxicol., 2016, 90, 2979. [2] W. R. Proctor et al., Arch. Toxicol., 2017, 91, 2849.

Microphysiological, organ-on-a-chip, systems (MPS) are emerging as a potentially more predictive model for human pathophysiology and toxicity. MPS often use human cells and media flow to model physiological stimuli in vivo. Due to the interest in these platforms, the Food and Drug Administration Foods Program partnered with Emulate to evaluate the utility of the Beta Human Liver Emulation System (BHLES) for the FDAA Foods Program’s regulatory science program. The BHLES is a MPS using flow and human liver cells (primary hepatocytes and liver sinusoidal endothelial cells) loaded onto a Liver-Chip. To test this system, Liver-Chips and traditional 24-well plates, for simultaneous comparison, were exposed to select compounds encompassing a range of hepatotoxic potential for 3 consecutive days. These compounds were known hepatotoxic compounds including uric acid, benzbromarone, tamoxifen, diglycolic acid, and acetaminophen and compounds that have no reported human cases of liver toxicity including dimethyl sulfoxide, theophylline, and aminophylline. Chemical toxicity was assessed by albumin secretion, urea release, LDH release, nuclear quantification, mitochondrial membrane potential, and apoptosis. System/platform performance was evaluated in terms of sensitivity and specific power, and variability and repeatability. Additionally, chemical interactions with the Chip material were assessed. Preliminary findings suggested that for the model test compounds selected, the BHLES was able to produce predictable results and performed similarly to 24 well plates. However, some compounds interacted with the Chip material altering the response. In general, the BHLES had high sensitivity and specificity. Only 3 Liver-Chips were necessary to detect a large effect for biochemical

Vehicle (DMSO). RES did not increase cell number as part of its protective mechanism. Mitochondrial function was decreased by cisplatin as evaluated using a Seahorse XFe Analyzer. Basal and maximal mitochondrial respiration were monitored in RES and cisplatin treated cells. Additional studies examined whether cisplatin and RES altered expression of mitochondrial complex proteins, biomarkers of mitophagy and oxidative stress. Western analysis detected an increase in mitophagy protein, cytochrome c, which was reversed by RES. RES protects human proximal tubular epithelial cells from cisplatin cytotoxicity, preserves mitochondrial integrity and mitochondrial respiration. Supported by NIH Grant INBRE BP20GM103434 and a WV NASA Undergraduate Research Fellowship to MD and RM.

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endpoints and 4 Liver-Chips were necessary to detect a very large effect for imaging endpoints. Finally, variability both within and between experiments was low. These results have begun to lay the groundwork for future use of MPS in a regulatory setting research. Further studies are necessary to fully understand the utility of the platforms in regulatory research environment.

2135 Exploring Pexidartinib-Induced Bioenergetic Alteration in Hepatic and Cardiac Cells Using the Seahorse Extracellular Flux Analyzer

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Approximately 60% of FDA-approved small molecule kinase inhibitors (KI) have identified mitochondrial liabilities in nonclinical investigations. However, these detrimental effects were mainly observed at high concentrations in isolated rodent mitochondria, necessitating further studies to establish the relevance of the mitochondrial liabilities in humans. The drug label for the recently approved KI pexidartinib contains boxed warnings for hepatotoxicity, including serious and potentially fatal liver injury. The mitotoxic potential of pexidartinib was assessed in hepatic and cardiac cells using the Seahorse extracellular flux analyzer. Primary human hepatocytes (PHHs) were cultured as monolayers and treated with pexidartinib at concentrations of 5 to 50 µM, corresponding to 0.25 to 2.5-fold human peak blood concentrations (C_max) achieved at the recommended therapeutic dose. Seahorse analysis was performed 1 hour after drug treatment. Cellular mitochondrial oxygen consumption rates were decreased by 35% to 25% in a donor-dependent manner, with statistically meaningful inhibition even at 5 µM, 4-fold lower than clinical C_max exposures. In contrast, there was little effect on the extracellular acidification rate, indicating that pexidartinib did not significantly affect the glycolysis pathway. Similar effects were observed in primary cardiac toxicity studies using the rat cardiomyoblast cell line H9C2. These results demonstrate that pexidartinib impairs cellular mitochondrial respiration at clinically relevant concentrations, which may contribute to the pathogenesis of pexidartinib-induced organ toxicity, particularly hepatotoxicity.

2136 Macrophage-Derived Extracellular Vesicles Regulate Concanaavalin A-Induced Hepatitis by Suppressing Macrophage Cytokine Production

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Acute liver failure is a clinical syndrome of severe hepatic dysfunction. Immune cells play an important role in acute liver failure. In recent years, the immunoregulatory function of extracellular vesicles (EVs) has been reported; therefore, it is inferred that EVs play a role in immune-mediated hepatitis. In this study, we investigated the immunoregulatory function of EVs in concanavalin A (Con A)-induced hepatitis. The mouse model was prepared by a single intravenous administration of 15 mg/kg Con A, in which there was a significant increase in the serum EVs number. In an in vitro study, the number of secreted EVs was also significantly increased in Con A-treated RAW264.7 cells, a macrophage cell line, but not in Hepa1-6 cells, a mouse hepatoma cell line. In an in vivo EVs treatment study, EVs from Con A-treated mouse serum and Con A-treated RAW264.7 cells suppressed inflammatory cytokine production in Con A-stimulated RAW264.7 cells. mRNA sequencing analysis showed that the expression of mmu-miR-122-5p and mmu-miR-148a-3p was commonly increased in these EVs and EVs-treated cells. The pathways enriched in the predicted mRNA target genes included inflammatory response pathways. The mRNA levels of the target genes in these pathways (mitogen-activated protein kinase, phosphoinositide 3-kinase/Akt and Rho/Rho-associated coiled-coil containing protein kinase pathways) were decreased in the EVs-treated cells. In an in vivo RNA interference study, the knockdown of liver RAB27A, an EVs secretion regulator, significantly exacerbated Con A-induced hepatitis. These data suggest that macrophage-derived EVs play an important role in Con A-induced hepatitis through immunoregulation.

2137 High-Throughput Assessment of Increased Chemical Toxicity Due to Hepatic Steatosis

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Hepatic steatosis alters native liver xenobiotic metabolism, impacts the bioactivation or detoxification of chemicals, and may alter sensitivity to chemical toxicity. Here, we modeled this state in a human hepatic cell culture model to assess the impact of steatosis on chemical toxicity in a quantitative and high-throughput manner. We induced steatosis in human hepatoma-derived cells, HepaRGs, by dosing maintenance media with 1 mM 1:2 oleic palmitic free fatty acid for 1 week. Cytochrome P450 (CYP) gene expression and metabolic activity (CYPs 1A1, 1A2, 2B6, 2C9, 2E1, 3A4) was significantly altered in the steatotic culture condition. Relative culture viability was determined by Cell Titer Glo (CTG), lactate dehydrogenase (LDH) release, and multiplexed fluorometric measurements of nuclear morphology using the Opera Phenix high-content screening (HCS) system. Naïve and steatotic HepaRGs cells were exposed to known hepatotoxicant (rotenone) over a 5-point dose range for 24 or 48 hrs. Rotenone toxicity (IC50) shifted from a baseline 0.64 µM to 0.48 µM in steatotic cells as measured by CTG, from 0.83 µM to 0.57 µM measuring LDH, and 0.80 µM to 0.62 µM using cell counts derived from HCS. Additional toxicity measures - including morphology, reactive oxygen species generation, and mitochondrial membrane potential - as well as additional chemicals known to be impacted by CYP-mediated metabolism are currently being assessed. The results of this study indicated that we can measure the impact of pre-existing conditions on environmental chemical toxicity, which will contribute to ongoing efforts to assess environmental exposure risks to susceptible subpopulations. This abstract does not necessarily reflect US EPA policy. Mention of trade names is not an endorsement or recommendation for use.

2138 Automation and Validation of the OrganoPlate LiverTox for Hepatotoxicity Detection

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Drug-induced liver injury (DILI) is one of the leading causes of market withdrawal in the pharmaceutical industry and poses a serious health risk to affected patients. Identification of hepatotoxic compounds in the preclinical phase of drug development is key to preventing DILI, however currently employed animal and two-dimensional (2D) in vitro models do not adequately predict human hepatotoxicity. Existing models suffer from many challenges including species differences, throughput limitations, and lot-to-lot variability of primary human hepatocytes. Here, we developed a functional 3D in vitro model of the human liver, the OrganoPlate LiverTox compatible with automated liquid handling and validated for hepatotoxicity screening. To build the model, up to 96 independent 3D perfused cultures were established on MIMETAS OrganoPlate 2-lane using automated liquid handling to seed cells, dose, collect media, and add assay reagents. For cell seeding, induced pluripotent stem cell-derived hepatocytes (iHep) in extracellular matrix were added to a microfluidic channel, following which endothelial and Kupffer cells were added to an adjacent channel to mimic the liver sinusoid. Characterization of the model revealed long-term hepatocyte function including CYP3A4 activity, as well as albumin and urea production for up to 14 days of culture. Fetal hepatocyte marker alpha-fetoprotein (AFP) dramatically declined over the 14 day culture, supporting iHep maturation in the OrganoPlate LiverTox. Assay validation studies using troglitazone as a positive hepatotoxic control compound revealed robust Z-factors >0.2 for albumin, urea, iHep viability (propidium iodide staining), and iHep nuclear size (Hoechst 33342 staining) assay readouts. Using these assays, 159 compounds of known hepatotoxicity were screened in the OrganoPlate LiverTox (50 µM, 72 h) and ranked by a composite score by combining the assay readouts. A follow-up dose response evaluation of select hits suggested the albumin assay to be the most sensitive readout in calculating TC50 values. Taken together, the OrganoPlate LiverTox is a promising platform for hepatotoxicity detection and has the potential to be used in a high throughput screening capacity.
New approach methodologies (NAMs) are gaining increasing importance as an alternative to traditional toxicity testing owing to high cost and time consumption required as well as ethical considerations for conducting in-life studies. Both regulatory agencies and industry are thus working towards developing biologically relevant in vitro cell models for efficiently and accurately predicting toxic liabilities of chemicals. The liver has been a major focus of these efforts, yet there are currently no in vitro alternatives for hepatotoxicity testing accepted by regulators. While hepatocytes are the primary component of the liver, the liver non-parenchymal cells (NPCs) also play an important role in hepatotoxicity. We have developed 2D and 3D in vitro models using co-cultures of primary rat hepatocytes and NPCs as an alternative to traditional toxicity testing. To assess the ability of these models to recapitulate rodent in vivo liver phenotypes we have tested the transcriptomic response of four hepatotoxins with well-known mechanisms of action (MOAs). The cultures were treated with gemfibrozil, GW7647, ketoconazole and phenobarbital for 1, 3 or 7 days. TempO-Seq analysis using BioSpyder’s Rat whole transcriptome panel was performed. We observed that each model system captured transcriptomic changes in gene expression consistent with the known MOA for these hepatotoxins. However, transcriptomic analysis of 2D mono- and co-culture models revealed that co-culture response to phenobarbital (10mM) is more representative (52% increase in similarity of the ontology enrichment patterns) (using existing in vivo data, 300mg/kg 24h). 3D rat culture models, whether mono- or co-culture, were found to be more consistent in their ontology enrichment patterns compared to 2D models. This was evident from the observation that the Modified Jaccard Index (MJI) scores between 3D mono-culture and co-culture models response to 30µM ketoconazole showed a 28.6% increase whereas the next best score of 0.522 between 2D mono-culture and co-culture pair. Ongoing detailed analysis of 2D and 3D co-culture systems seeks to determine the optimal system for capturing response to chemical exposure that is most representative of in vivo response.

We have previously demonstrated alterations in hepatocyte-derived exosomes (HDEs) prior to and in the absence of overt necrosis associated with idiosyncratic drug-induced liver injury (IDILI). HDEs contain mRNAs, miRNAs, and proteins which may possess value in the form of sensitive and specific biomarkers for IDILI liability. The objective of this project was to identify HDE-based biomarkers of IDILI by profiling protein changes in primary human hepatocytes from N-5 donors exposed to subtoxic and toxic concentrations of the IDILI drugs tolcapone, isoniazid, and bosentan, which were selected to represent different mechanisms of injury. After a 24 h exposure, HDEs were enriched from culture medium by ultracentrifugation and changes in exosomal proteins were assessed using global proteomic profiling. Significance was determined by an ANOVA model with linear contrasts between each compound concentration and DMSO control (p<0.05 and fold change>1.5). Three proteins: complement factor 1 (C1F), inter-alpha-trypsin inhibitor heavy chain 1 (ITIH1) and valosin-containing protein (VCP) were significantly altered in response to both concentrations of all IDILI compounds; C1F and ITIH1 were significantly decreased whereas VCP was significantly increased. All 3 proteins were readily quantifiable in HDEs by ELISA. ITIH1 was selected for further validation, as previous studies demonstrate a decrease in ITIH1 in DILI, it is liver-specific, and our profiling data supports its consistent responses across all 5 hepatocyte donors. In validation studies, concentration-dependent decreases in exosomal ITIH1 quantified by ELISA were observed in IDILI-drug treated hepatocytes from additional hepatocyte donors prior to a significant decrease in ATP. Interestingly, exosomal ITIH1 was negatively correlated with decreased ATP. Interestingly, exosomal ITIH1 was negatively correlated with mitochondrial dysfunction and oxidative stress were quantified on a population as well as single cell level. Oral equivalent doses were extrapolated using physiologically based pharmacokinetic modeling along with reverse dosimetry. Chemicals induced a concentration-dependent increase in lipid accumulation, disruption of mitochondrial function and integrity, and increase in oxidative stress, including heterogenous responses of single cells in the population. Extrapolated oral equivalent doses for the key events addressed were within biologically relevant exposure ranges for the test chemicals. Future research will aim to establish a predictive model and screen many food-related chemicals for their risk to induce NAFLD.
2143 Pharmacological Intervention of Gilbenclamide and Dimethyl Fumarate against Thioacetamide-Induced Liver Fibrosis in Mice: Comparative Study with MCC950 and 4 Octyl Itonaconate


Chronic tissue injury leads to fibrosis of multiple organs, responsible for one-third of the death, worldwide. Liver fibrosis is a common condition involved in several types of chronic liver diseases like cirrhosis and hepatocellular carcinoma. Thioacetamide (TTA) has been reported to precisely mimics histological characteristics of initiation and progression of human liver fibrosis in rodents. Anti-fibrotic agents like Gilbenclamide (GLB) and Dimethyl Fumarate (DMF) have demonstrated promising preventive properties and inhibits NLRP3 inflammasome activation. Dimethyl fumarate (DMF), a multiple sclerosis drug, activates the Nrf2/ARE pathway and maintains the antioxidant status in the cell. The present study was aimed to evaluate the hepatoprotective effects of GLB (gilbenclamide) and DMF on the experimental TAA-induced liver fibrosis.

2144 PPARα Agonist WY-14,643 Escalates Blood Alcohol Clearance but Promotes Liver Injury in Ethanol/Nicotine-Fed Mice

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Alcohol consumption induces fatty liver. Peroxisome proliferator-activated receptor α (PPARα), a fatty acid oxidation regulator, inhibits alcohol-induced fat accumulation in liver, i.e., alcoholic fatty liver (AFL). Alcohol drinkers frequently smoke tobacco and E-Cigars. Previously we reported that nicotine enhances AFL. In this study, we investigate whether PPARα activation also blocks nicotine-enhanced AFL. Nicotine-induced Lieber-DeCarli ethanol liquid diet to induce AFL, nicotine was added to ethanol diet to induce nicotine-enhanced AFL, and PPARα agonist WY-14,643 was added to the above diets at 1 mg/L, which is much lower than usually used dose of 0.1% (1 g/L liquid diet or 1 g/kg solid chow). The mice were fed the diets for 3 weeks. The results showed that at such a much lower dose, WY-14,643 still blunted AFL and nicotine-enhanced ALC, which was paralleled with induction of catalase and fatty acid oxidation-related enzymes such as CYP4A11, acyl-CoA oxidase (ACOX), and liver fatty acid binding protein (L-FABP). The low dose of WY-14,643 still induced hepatomegaly, either in ethanol/WY-14,643 group or nicotine/ethanol/WY-14,643 group. Serum ALT was dramatically increased by the ethanol/WY-14,643 feeding and was further increased by nicotine/ethanol/WY-14,643 feeding, which was confirmed by pathology H&E staining in liver sections. Interestingly, serum alcohol levels were dramatically decreased by WY-14,643. Ethanol is mainly metabolized by alcohol dehydrogenase (ADH), CYP2E1 and catalase, but ADH was not changed and CYP2E1 were decreased by WY-14,643, indicating that the induced catalase might be responsible for the escalated blood alcohol clearance. To further confirm the escalated alcohol clearance effect of WY-14,643, mice were treated ethanol by gavage at 5 g/kg after a 3-week feeding in the presence or absence of WY-14,643. Serum ethanol levels at 8 h were much lower in WY-14,643 group than in the control group. WY-14,643-induced alcohol clearance, however, ethanol-induced liver injury were not observed in mice lacking PPARα, which confirms that WY-14,643 exerts effects via PPARα. As a target of PPARα, L-FABP was not involved in the alcohol clearance since WY-14,643-induced alcohol clearance was still observed in the mice lacking L-FABP. In conclusion, WY-14,643 speeds up alcohol clearance via induced catalase, which along with induced lipid metabolism enzymes protects against AFL. However, whether the liver injury deterioration is associated with the extremely quick ethanol oxidation by catalase needs further studies.

2145 Single Cell RNA Sequencing Reveals Downregulation of lncRNA Gm42031 Expression after Treatment with AhR Ligands in Concanavalin-Induced T Cell–Mediated Liver Injury

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Long-chain non-coding RNAs (lncRNAs) have been implicated in many biological processes and have been shown to have abnormal expression in inflammatory reactions and diseases. Recent studies have been exploring these non-coding RNAs to further elucidate their relationship with inflammatory diseases. Data from our lab has been generated that indicates that AhR Hydrocarbon Receptor (AhR) activation by 2,3,7,8-Tetrachlorodibenzop-dioxin (TCDD) triggers dysregulation in epigenetic pathways. Further, we have shown that this environmental pollutant promotes the differentiation of Fox3+ regulatory T cells (Tregs) while an endogenous AhR ligand, 6-formylindolino[3,2-b] carbazole (FICZ), exerts contrasting effects and promotes proinflammatory Th17 cells. In an effort to further explore activated inflammatory genes altered upon treatment with these ligands, a murine model of immune cell-mediated liver injury was employed by intravenously injecting 12.5 mg/kg Concanavalin A (ConA), a polyclonal T cell mitogen. Mice were treated one hour after challenge with vehicle, 10 μg/kg TCDD, or 50 μg/kg FICZ intraperitoneally. Single cell RNA sequencing (scRNA-seq) was conducted on infiltrating liver mononuclear cells enriched via a percoll gradient. Upon analysis, immune cell clusters were identified and included multiple B cell and T cell clusters, as well as Kupffer cells, neutrophils, NK cells, among others. We observed a clear increase in the number of T cells and a decrease in the number of Kupffer cells, fibroblasts, and neutrophils upon TCDD treatment. Additionally, Gm42031 was identified as an lncRNA upregulated in vehicle-treated ConA challenged mice but downregulated in both AhR ligand-treated groups, specifically in the B cell, CD8+ T cell, Kupffer cell and NK cell populations. For the first time, this data suggests Gm42031 as a potential player in liver inflammation that was downregulated following AhR ligation. Supported by NIH grants R01AT039961, P20GM103641, R01ES030144, R01AI27978 and R01AI23547.
Acetaminophen (APAP) overdose is the main cause of acute liver failure in the US. APAP hepatotoxicity is associated with a sterile inflammatory response that is characterized by the infiltration of neutrophils and monocytes after liver injury. While the contribution of these immune cells to promote liver repair has been demonstrated, mechanisms involved in cross-talk between immune cells and hepatocytes to help facilitate hepatocyte proliferation after APAP overdose remain unclear. The purpose of this study was to investigate this relationship between immune cells and hepatocytes with a focus on the chemokine receptor CXCR2. C57BL/6J mice were subjected to an APAP overdose (300mg/kg) and the role of CXCR2 in mediating liver recovery was investigated using the CXCR2 inhibitor SB225002 (4mg/kg). Additionally, clodronate liposomes were used to deplete macrophages prior to APAP treatment and the influence of this on CXCR2 expression was assessed. Isolated primary mouse hepatocytes (PMH) were also used for confirmatory experiments. Our data show that CXCR2 is mainly expressed on hepatocyte membranes and is induced specifically in cells around the necrotic areas 24 hours after APAP. Inhibition of CXCR2 with SB225002 caused a delay in shrinkage of necrotic areas and a decrease in hepatocyte PCNA expression indicating its importance in the regenerative response after APAP, which is mediated through the AKT signaling pathway within hepatocytes. The role of AKT signaling in CXCR2 mediated liver recovery was confirmed in PMH by treatment with MIP-2, a CXCR2 ligand. Depletion of Kupffer cells prevented CXCR2 induction on hepatocytes by 24 hours after APAP, which also correlated with a significant decrease of IL-1β and IL-10. Our data demonstrate the important role of CXCR2 in mediating hepatocyte proliferation through activation of AKT signaling to facilitate liver repair after APAP, which is regulated by resident macrophages, potentially by release of IL-10 or IL-1β.

2147 Kupffer Cells Regulate Liver Recovery through Induction of Chemokine Receptor CXCR2 on Hepatocytes after Acetaminophen Overdose in Mice
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2148 Spatial Reconstruction of the Early Hepatic Transcriptomic Landscape after an Acetaminophen Overdose Using Single Cell RNA Sequencing
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Metabolic zonation in the liver involves the spatial separation of different metabolic pathways along the portal-central axis. The prototypic feature of metabolic zonation is enhanced oxidative phosphorylation in periportal (PP) hepatocytes and greater drug metabolism and detoxification in pericentral (PC) hepatocytes. We were interested in leveraging single-cell RNA sequencing to study how the spatial composition of the liver is dysregulated following an overdose of acetaminophen (APAP), which is the leading cause of drug-induced liver injury in the United States. We treated mice with 300 mg/kg APAP and isolated hepatocytes 2h later to study the initial transcriptomic response at a single-cell resolution before overt cell death. In order to reconstruct the early transcriptomic APAP liver lobule, we spatially profiled single hepatocytes along a linear axis from the portal vein to central vein based on key landmark genes. A hallmark characteristic of APAP overdose is centrilobular necrosis which suggests that PC hepatocytes respond to APAP differently than PP hepatocytes. By creating a spatial map of both the untreated and APAP-treated liver lobules, we had the unprecedented ability to compare APAP hepatocytes from anywhere along the zonation axis to the respective control hepatocytes (e.g. APAP PC hepatocytes vs control PC hepatocytes), effectively negating the inherent spatial differences in gene expression. We found that many genes are exclusively induced within pericentral hepatocytes and remain unchanged in periportal hepatocytes. Pathway analysis revealed that these genes were related to translation, oxidative phosphorylation, DNA damage, glutathione metabolism and mitogen-activated protein kinase signaling. Additionally, we identified a stress program for hepatocytes directly surrounding the central vein that culminated in cell death. Lastly, we spatially ordered human liver scRNA-seq data along the portal-central axis validating our liver lobule reconstruction in human hepatocytes.

2149 A Retrospective Analysis of Hepatocyte Hypertrophy in Repeated-Dose Rat Studies

Hepatocyte hypertrophy is generally considered an adaptive change of the liver which reflects compromised cytochrome P450 enzyme induction, which sometime results in decreased systemic exposure of the active compound. This is more often observed in nonclinical studies as relatively higher doses are administered to animals to investigate the toxicity of the test article and establish sufficient safety window for clinical trials. Strong enzyme inducers are also of regulatory concern in terms of drug approval. The purpose of this investigation is to analyze the hepatocyte hypertrophy observed in rat studies conducted at the facility and provide references as background data. It was also intended to analyze if there are any relationships between hepatocyte hypertrophy and accumulation index of systemic exposure. A retrospective analysis was performed on approximately 150 repeated dose rat studies of 4-week to 26-week duration tested with non-biologics. The animals used on studies were Sprague Dawley and Wistar rats sourced from approved sources. The results showed that hepatocyte hypertrophy was noted in approximately 14% studies and represents approximately 14% of total compounds tested. The hepatocyte hypertrophy was either in centrilobular or diffuse pattern, of minimal to moderate severity, and was accompanied with increased relative liver weight (to body weight) by 12% to 98% relative to the concurrent control. There were no associated changes in ALT or AST or the increases were of low magnitude and were not considered adverse. In 12 of the 21 studies, hepatocyte hypertrophy was the only test article-related change observed in the liver. Other liver changes seen in these studies included hepatocellular vacuolation and/or necrosis. When hepatocyte hypertrophy (HH) was observed along with follicular hypertrophy in the thyroid glands, which is another indicator of enzyme induction, these tend to be associated more with decreased exposure and greater magnitude of increased liver weights. The HH was revealed in all studies analyzed expect that in 2 studies it was still noted as a minimal or mild change in one animal following a 2-week recovery or only in the high dose group following a 4-week recovery. For two compounds that both the IND enabling and longer term studies were conducted at WuXi, HH was noted in both the 4-week and 13- or 16-week studies. Among the 19 compounds that resulted in HH only three compounds also had similar reversible changes in the non-rodent species. In most studies, the HH by itself was not considered an adverse change.

2150 A Novel Gene Expression Profiling Approach in Collaborative Cross Mice Identifies Mechanisms and Risk Factors Contributing to TAK-875-Induced Liver Injury
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Development of TAK-875 was discontinued when a small number of serious drug-induced liver injury (DILI) cases were observed in Phase 3 clinical trials. Previous studies have suggested that the mechanisms of TAK-875 DILI are multifactorial and that the test article may contribute to toxicity susceptibility in patients. In this study, we tested the hypothesis that gene expression profiling in the Collaborative Cross mouse population after an acute high-dose exposure could also be used to inform early liver events and identify genetic risk factors contributing to TAK-875 hepatotoxicity. Eight (8) male mice (4 matched pairs) from each of 45 Collaborative Cross strains were treated with a single oral (gavage) dose of either vehicle or 600 mg/kg TAK-875. Liver injury was not detected by histological change after the acute high-dose exposure. However, statistically significant treatment-induced alterations were observed in plasma total bile acids at 6-h post dose across nearly all the Collaborative Cross strains. Plasma total bile acid levels correlated with treatment-induced alterations in alanine aminotransferase at 24-h post dose, indicating strain-dependent differences in susceptibility to early TAK-875-induced hepatocellular stress. Gene expression profiling in the liver identified treatment-induced changes that differed by strain and correlated with changes in serum liver chemistries. The affected pathways included mitochondrial dysfunction, oxidative stress, bile acid homeostasis, and immune responses—mechanisms reported to contribute to TAK-875 toxicity in previous studies. Candidate risk factor genes contributing to these responses were then identified using a novel pathway-based approach and individual-variant enrichments reflected pathways enriched among genes associated toxicity susceptibility in DILI patients. Taken together, these findings demonstrate a novel preclinical approach that identified mechanisms and risk factors underlying TAK-875 toxicity and that may be helpful in understanding, predicting, and ultimately preventing DILI for other drugs.
Acetaminophen (APAP)-induced acute liver injury/failure (ALI/ALF) in patients is associated with marked changes in the coagulation system, particularly in ALF. However, standard experimental APAP overdose in mice is followed by resolution of liver injury, not progression to liver failure, limiting discovery of the underlying mechanisms of coagulopathy when the disease progresses. The aim of this study was therefore to define the state in experimental APAP-induced ALI and APAP-induced ALF. Wild-type C57BL/6J mice were challenged with various hepatotoxic doses of APAP (300 mg/kg, 450 mg/kg or 600 mg/kg, i.p.) or vehicle (saline), and liver and blood were collected 24h after challenge. Plasma alanine aminotransferase (ALT) levels increased in a dose-dependent manner in APAP-challenged mice. The amount of centrilobular necrosis was similar at all APAP doses. However, a clear increase in hemorrhage/congestion and vacuolization of hepatocytes distinguished mice challenged with 600 mg/kg APAP. We observed dose-dependent effects of APAP on body temperature (rectal) at 24h, with severe hypothermia (i.e. < 30°C) evident at the 600 mg/kg dose. Liver function, as assessed by plasma albumin and direct bilirubin levels, was dramatically decreased in mice receiving 600 mg/kg APAP compared with lower APAP doses and vehicles. Whereas biomarkers of liver injury/function displayed a more uniform dose-response, more dramatic changes in biomarkers of coagulation were observed between 450 mg/kg and 600 mg/kg APAP. Biomarkers of coagulation cascade activation (i.e., thrombin anti-thrombin complexes and d-dimer) were dramatically elevated in mice given 600 mg/kg. Whereas prothrombin time (time to clot) was slightly prolonged in mice challenged with 300 or 450 mg/kg, most plasma samples from mice challenged with 600 mg/kg APAP failed to clot at all. In vivo thrombin generating capacity was significantly reduced in all three APAP groups compared with vehicle. The results indicate that profound changes in the coagulation system are observed in mice challenged with APAP at doses producing features of liver failure. These results suggest the possibility that experimental APAP overdose can be used to uncover the mechanistic basis of coagulation changes observed in ALI and ALF patients.
Renal Transporter Expression Changes in Rodent Models of Nonalcoholic Steatohepatitis

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Inter-individual alterations to renal elimination processes can lead to adverse drug reactions. Nonalcoholic steatohepatitis (NASH) is known to alter hepatic drug transport but may also affect renal transporters as well. This study investigates renal physiological changes in rodent models of NASH for identification of a model that recapitulates human alterations. Rats on methionine and choline deficient (MCD), atherogenic (Athero) or control; Lepr(db/db) mice on a high fat diet (HFD); and Lepr(db/db) mice on fast for two days (FFDTH), American lifestyle induced obesity syndrome (ALIOS) or control diet were used to characterize hepatic and renal pathology, clinical chemistry, and the transcriptional and translational expression of drug transporters by rTaqPCR and surrogate peptide LC-MS/MS, respectively. Renal pathology confirms development of NASH while renal pathology demonstrates no lesions except mild lipid accumulation in ALIOS. MCD, Athero and ALIOS exhibit an increase in blood urea nitrogen while all models, except Athero, demonstrate a decrease in GFR. There is a significant decrease in protein expression for renal basolateral uptake transporters, OCT3 and OATP4C1, for mouse models (from 1.97, 0.67 to 1.17, 0.35 db/db; 1.43, 0.27 FFDT; 1.50, 0.34 ALIOS pmol/mg protein, respectively) but no other rat model. Similarly, renal apical uptake transporters, OAT5 and OATP1A1, exhibit a significant decrease in mouse models (from 4.59, 0.69 to 0.45, 0.02 db/db; 1.59, 0.21 FFDT; 2.83, 0.36 ALIOS, respectively) but a significant increase of OAT5 in MCD (1.67 to 4.17 pmol/mg protein). Renal apical efflux transporters elicit a variety of significant changes in protein expression including an increase of MRP2 in MCD (0.17 to 0.30), decrease of BCRP in db/db and ALIOS (from 0.25 to 0.14, 0.13, respectively) and a decrease of MRP4 in mouse models (from 0.87 to 0.34 db/db; 0.29 FFDT; 0.18 ALIOS pmol/mg protein). The only significant change in renal basolateral efflux transporter expression is a decrease in MRP1 protein in MCD and Athero (from 2.71 to 1.53, 1.68 pmol/mg protein, respectively). These data suggest variations in renal physiology are elicited by NASH in a range of rodent models. This demonstrates the potential variability in renal elimination of drugs during NASH and provides a resource to identify the appropriate model for future studies.

Late Protective Effects of Adenosine A2B Receptor Activation in Acetaminophen Hepatotoxicity

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Acetaminophen (APAP) overdose is the most common cause of drug induced hepatotoxicity and acute liver failure (ALF) in the United States. Though the current antidote, N-acetyl cysteine (NAC) is very effective when administered early after an APAP overdose, most patients present late to the clinic, when NAC efficacy is declining. Thus, there is an urgent need for novel late acting therapeutic options which can prevent progression to ALF. We have demonstrated earlier that the neuronal guidance cue netrin-1 could attenuate APAP hepatotoxicity in a delayed fashion by promoting liver recovery and regeneration mediated through the Adenosine A2B receptor (A2BAR). In the present study, we demonstrate the clinical relevance of this mechanism by pharmacological intervention targeting A2BAR after an APAP overdose in vivo in comparison to the standard of care, NAC. Male C57BL/6J mice were treated with 300 or 600mg/kg APAP, followed by vehicle control (DMSO) or the A2BAR agonist Bay-606583 administered 1.5 or 6 hours later. Mice were sacrificed at 6 or 24 hours for assessment of APAP-induced cellular signaling or liver injury. Bay-606583 treatment 1.5 hours after APAP overdose (300mg/kg and 600mg/kg) was found to provide significant protection against liver injury at the 6h time point by preserving mitochondrial function despite JNK activation and its mitochondrial translocation. This protection by A2BAR activation was sustained when Bay-606583 was given 6h after APAP overdose, a time point at which NAC administration did not show benefit. Flow cytometry analysis indicated that activation of the A2B receptor through Bay-606583 treatment 6 hours post APAP resulted in enhanced infiltration of macrophages with the reparative (M2) phenotype by 24 hours, accompanied by a decrease in neutrophil infiltration. Thus, our data emphasize the protective effect of activating A2BAR after an APAP overdose with attenuation of mitochondrial dysfunction when administered early and probably facilitates liver regeneration when administered in a delayed fashion, long after the standard of care NAC ceased to be effective.

Hyaluronan Synthesis Inhibition Normalizes Hepatic Fibrogenic Features by Reducing Hepatic Stellate Cell Activation in Alcohol-Associated Liver Disease Models

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Ethanol is a toxin and its over consumption promotes alcohol-associated liver disease (ALD). Hyaluronan (HA), an extracellular matrix glycosaminoglycan, is increased in livers and blood of advanced liver disease patients who consume alcohol. Hepatic stellate cells (HSCs) are the major liver HA producers, and HA synthesis is required for fibroblast to myofibroblast transition in extrahepatic organs. Further, increased HA content and fragmentation drive inflammation and fibrosis in many tissues. Hence, treatment of alcoholic liver disease (ALD) has been hypothesized that ethanol enhances HSC activation through hyaluronan production in early ALD. Mice were fed a 2% (v/v) ethanol-containing diet or control diet for 2 days after which they were given a single carbon tetrachloride (CCL4) injection. To inhibit HA synthesis, we provided 4-methylumbelliferone (4MU, 450 mg/kg orally), daily. We used LX2 cells to determine the impact ethanol (50 mM, 48h) had on LPS (100 ng/mL) responses, with or without concurrent 4MU treatment. Ethanol feeding enhanced CCL4-induced hepatic HA accumulation, this was reduced by 4MU treatment. CCL4 exposure increased hyaluronan synthase (Has1 and Has2 expression but, only Has2 transcripts were enhanced by ethanol feeding; Has2 transcripts were normalized by 4MU treatment. Acta2 and Ccl2 transcripts were greater in ethanol-fed mice after CCL4 and 4MU treatment normalized this. Although induced by CCL4, ethanol feeding did not enhance Tnf transcripts. Ethanol-exposed LX2 cells made more Ccl2 mRNA after LPS treatment when compared to LX2 cells not exposed to ethanol. 4MU suppressed ethanol-enhanced Ccl2 expression by LX2 cells. Finally, studies with human liver tissue microarrays indicated that HA and collagen
content were greater in steatotic livers from donors who consumed alcohol when compared to those who did not. In conclusion, these data suggest that ethanol exposure enhances HSC activation through HA production. Inhibiting HA production by HSC may therefore attenuate liver disease progression in ALD patients. Supported by P20GM103549 & P30GM118247.

2159 A Non-mitogenic FGF1ΔHBS Variant Protects from Nonalcoholic Fatty Liver Disease via Activating AMPK-Mediated Pathways

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Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disorder. Fibroblast growth factor 1 (FGF1) demonstrated protection against NASH in type 2 diabetic and obese mice by an uncertain mechanism, and its strong mitogenic activity limits its potential clinical application. Our recently engineered FGF1 variant (FGF1ΔHBS) greatly reduces mitogenic proliferative potential, while preserving the full metabolic activity of wild-type FGF1. We investigated the therapeutic activity and mechanism of FGF1ΔHBS against NAFLD in the present study. FGF1ΔHBS administration was effective in 9-month-old db/db mice with NAFLD; liver weight, lipid deposition and inflammation declined, and liver injury decreased. FGF1ΔHBS reduced oxidative stress by stimulating nuclear translocation of nuclear factor erythroid 2-related factor 2 (Nrf2) and elevation of antioxidant protein expression. FGF1ΔHBS also inhibited activation and/or expression of lipogenic genes, coincident with phosphorylation of AMP-activated protein kinase (AMPK) and its substrates. Mechanistic studies on palmitate exposed hepatic cells demonstrated that NAFLD-like oxidative damage and lipid accumulation could be reversed by FGF1ΔHBS. In palmitate-treated hepatic cells, siRNA knockdown of Nrf2 abolished only FGF1ΔHBS anti-oxidative actions but not improvement of lipid metabolism. In contrast, AMPK inhibition by pharmacological inhibitor or siRNA abolished FGF1ΔHBS benefits on both oxidative stress and lipid metabolism that were FGF receptor 4 (FGFR4) dependent. Further support of these findings is that liver-specific AMPK knockout abolished therapeutic effects of FGF1ΔHBS against high-fat, high-sucrose diet-induced hepatic steatosis. Moreover, FGF1ΔHBS improved high-fat/high-cholesterol diet-induced steatohepatitis and fibrosis in apolipoprotein E knockout mice. FGF1ΔHBS decreased the liver weight, lipid deposition, fibrosis, inflammation and ameliorated the liver injury, coincident with the upregulation of the phosphorylation of AMPK and its substrate. These findings indicate that FGF1ΔHBS is effective for preventing and reversing liver steatosis and steatohepatitis and acts by activation of AMPK via hepatocyte FGF4. FGF1ΔHBS might be a therapeutic approach for the treatment of NAFLD without promoting undesired tissue hyperproliferation.

2160 Interaction of Environmental Vinyl Chloride Exposure and Diet: Potential Role of the Epitranscriptome

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Obesity as the primary factor of nonalcoholic fatty liver disease (NAFLD) doesn’t completely drive the overall interindividual risk for the development of severe NAFLD. In addition to genetic variation, risk may also be driven by environmental exposures, e.g. vinyl chloride (VC). At high exposure levels VC directly causes liver disease and cancer. However, we and others have shown that lower exposure levels (<OSHA limit) that are currently considered ‘safe’ exacerbate underlying liver disease and have been linked with human liver diseases. C57BL/6J mice were fed Western diet (WD), or low-fat control diet (CD) for up to 1 year. During the first 12 weeks of feeding, mice were also exposed to VC on at concentrations below the current OSHA limit (<1 ppm) or room air for 6 hrs/d, 5 d/wk. Plasma and liver samples were collected (during and after VC) for determination of injury and of chemical modifications on RNAs via LC-MS. Early changes due to VC exposure include dysregulated energy homeostasis and mitochondrial dysfunction - even in the absence of WD. In toto, VC limits the bioenergetic reserve capacity of the liver and exacerbates metabolic stress caused by obesity. Late changes include an increased number of tumors, ranging from moderately to poorly differentiated HCC. Interestingly, although VC significantly altered expression of several key metabolic regulatory proteins in the liver, these changes were not reflected at the level of steady-state mRNA expression. In contrast, the expression of several key epitranscriptomic modulators (e.g., Rbm15, Wtap, Kiaa1429 and Ythdf1) were altered by VC. Given that VC is well known to attack nucleic acids (e.g., DNA), that VC may be mediating this discord between mRNA and protein expression by also altering mRNA is distinctly possible. Indeed, a total of 29 modified nucleosides were significantly altered in mouse liver by VC and/or diet. We hypothesize that epitranscriptomic regulation of these target RNAs may contribute, at least in part, the observed disconnect between protein/activity and mRNA expression under our conditions. Taken together, our data indicate that VC sensitizes the liver to other stressors (e.g., WD) resulting in enhanced tumorigenesis. These data emphasize that current OSHA safety restriction may be insufficient to account for other factors that can influence hepatotoxicity in humans.

2161 Welding Fume Inhalation Exposure and High-Fat Diet Change Lipid Homeostasis in Rat Liver


It is estimated that greater than one million workers are exposed to welding fume (WF) by inhalation daily. The potentially toxic metals found in WF are known to cause multiple adverse pulmonary and systemic effects, including cardiovascular disease, and these metals have also been shown to translocate to the liver. This occupational exposure combined with a high fat (HF) Western diet, which has been shown to cause hyperlipidemia and non-alcoholic fatty liver disease (NAFLD), has the potential to cause significant mixed exposure metabolic changes in the liver. Matrix assisted laser desorption ionization (MALDI) mass spectrometry allows for direct analysis of tissues for the identification and relative quantification of multiple biomolecules, including lipids. The goal of this study was to use matrix assisted laser desorption ionization imaging mass spectrometry (MALDI-IMS) to analyze the spatial distribution and abundance changes of lipid species in Sprague Dawley rat liver maintained on a HF diet combined with WF inhalation. Male Sprague Dawley rats were exposed to VC through inhalation to a target concentration of 20 mg/m3 for 3 hours per day for 5 weeks or filtered air as the control. The results of the MALDI-IMS analysis revealed unique hepatic lipid profiles for each treatment group at 12 weeks post-exposure. Pulmonary exposure to WF alone increased the levels of ceramide-1-phosphate and lysophosphatidyl ethanolamine (LPE) which are both markers of inflammation. The HF diet group had significantly increased abundance of triglycerides and phosphatidyl ethanolamine lipids, as well as decreased lysophosphatidic lipids and cardiolipin. The increased hepatic triglycerides were found in conjunction with significantly increased serum triglycerides and oil-red-O staining showed increased lipid deposition in the HF diet animals. Ceramide-1-phosphate was found at higher abundance in the regular (REG) diet WF-exposed group which has been shown to regulate the eicosanoid pathway involved in pro-inflammatory response. The results of this study showed that the combined effects of WF inhalation and a HF diet significantly altered the hepatic lipoprotein.

2162 Biliary Iron Excretion in Slc30a10- and Slc39a14-Deficient Mouse Models

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In diseases of iron (Fe) overload, the liver accumulates Fe resulting in fibrosis, cirrhosis, and carcinomas. Fe is believed to be eliminated by the body by sloughing of intestinal epithelium and other passive means. However, Fe can also be excreted into bile, although the mechanisms and relevance to Fe homeostasis are not known. In the current study, we investigated biliary Fe excretion using Slc30a10 and Slc39a14 knockout mouse models via a dietary approach. Wild-type and mutant mice were raised on Fe-sufficient and -rich diets for one month and subject to surgical collection of bile followed by organ harvest. Liver and biliary Fe levels were decreased in Slc39a14-deficient (10-fold in liver, 13-fold in bile) mice raised on the Fe-rich diet but not in Slc30a10-deficient mice raised on the Fe-rich diet. Histopathological examination using Prussian Blue Fe stain identified Fe accumulation primarily in hepatocytes of Slc30a10-deficient mice and in extrahepatic cells in Slc39a14-deficient mice. Mass spectrometric analysis of bile from wild-type mice raised on a Fe-rich diet showed a significant changes in relative abundance of ferritin and other proteins. We are currently investigating the biochemical form of Fe excreted in the bile. Results from these experiments provide insights into novel molecular targets that can be further explored to increase biliary Fe excretion and reduce total Fe burden in the diseases of Fe excess and toxicity.
In severe cases of acetaminophen (APAP) overdose, acute liver injury rapidly progresses to acute liver failure (ALF), producing life threatening cardiac instability, hepatic encephalopathy, and multi-organ failure. Systemic effects of the anti-inflammatory cytokine interleukin-10 (IL-10) are highest in ALF patients with the poorest prognosis. The mechanism-based for this counter- intuitive connection is not known, as elevation in IL-10 levels is hypothesized to counteract the pathologic effects of inflammatory cytokines. In mice challenged with 300 mg/kg APAP, we found that hepatic necrosis developed concurrently with systemic elevation in proinflammatory cytokines. As anticipated, resolution of liver injury in this experimental setting was linked to trafficking of monocyte-derived macrophages (MDMs) into necrotic areas, a shift towards pro-repair macrophage phenotype, and termination of pro-inflammatory cytokine expression. By contrast, in mice treated with 600 mg/kg APAP, a challenge that recapitulates many of the features of APAP-induced ALF in mice, intrahepatic trafficking of MDMs was disrupted resulting in a persistence of dead cell debris. Further, as in ALF patients, levels of proinflammatory cytokines and IL-10 were dramatically elevated in these mice. Interestingly, in this experimental setting, Kupffer cells, the resident macrophages of the liver, exhibited many characteristics of myeloid-derived suppressor cells, including high-level expression of IL-10. In stark contrast to the widely held dogma that IL-10 is beneficial in this setting, inhibition of IL-10 completely restored macrophage trafficking in the livers of mice with ALF. Likewise, pharmacologic elevation of systemic IL-10 levels in mice given 300 mg/kg APAP dramatically impaired macrophage trafficking and inhibited resolution of liver injury. Collectively, the results indicate that exaggerated IL-10 expression disrupts intrahepatic macrophage trafficking and pro-repair polarization essential for liver repair. These are the first studies to document a mechanistic basis for the link between high IL-10 levels and poor outcome in patients with ALF.

**2163 Exaggerated Interleukin-10 Expression Inhibits Macrophage-Dependent Liver Repair in Acetaminophen-Induced Acute Liver Failure**


**2164 Diet-Induced Nonalcoholic Fatty Liver Disease Slowed Recovery of Hepatic Fibrosis and Carcinogenic Reprogramming after Microcystin-LR Toxicity in Rats**

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Continual exposure to exogenous stressors such as poor diet or toxins/toxicants result in various metabolic disorders, including the progressive liver disease nonalcoholic fatty liver disease (NAFLD). NAFLD causes liver extracellular matrix remodeling that is an important risk factor for fibrosis and hepatocellular carcinoma (HCC). Microcystin-LR (MCLR) is a hepatotoxin produced by fresh-water cyanotoxins and has been shown to cause a NAFLD-like phenotype. Multiple preclinical studies indicate that MCLR causes liver fibrosis, and epidemiological studies further indicate that MCLR is a risk-factor for HCC. The current study investigated the hepatic recovery and potential carcinogenic effects of MCLR-elicted liver injury in pre-existing NAFLD. Male Sprague-Dawley rats were divided into two groups and fed either a control or a high fat/high cholesterol (HFHC) diet for eight weeks. Animals were then subdivided into two treatment groups receiving intraperitoneal injection of either saline or 300 mg/kg MCLR, one day for 2 weeks. Mice from each group were euthanized at one of three time points: at the completion of the MCLR exposure period, after 2 weeks of recovery, and after 4 weeks of recovery. Animals continued their respective diets throughout the study. Histology results show that after four weeks of recovery the MCLR-exposed HFHC animals had less steatosis and more fibrosis compared to the vehicle-exposed HFHC animals and MCLR-exposed control animals. RNA-Seq analysis demonstrated dysregulation of ECM genes (e.g., collagens, ECM regulators etc.) after MCLR exposure in both control and HFHC groups but persisted only in the HFHC groups at 2 and 4 weeks of recovery. KEGG pathway analysis indicated a significant upregulation in genes associated with “pathways in cancer”, “cell cycle”, and “microRNAs in cancer” in both control and HFHC diet groups after MCLR exposure. After 4 weeks of recovery, MCLR hepatotoxicity in pre-existing NAFLD persistently dysregulated genes related to cellular differentiation (e.g., brain-expressed X-linked 1 and carboxypeptidase A) and HCC (e.g., podoplanin and mesothelin). These data suggest that continued stress of a poor diet after MCLR exposure impairs hepatic recovery mechanisms and has the potential to contribute to NAFLD-associated HCC.

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**2165 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) Elicits Changes in Gut Microbiome Consistent with Progression of Steatosis to Steatohepatitis with Fibrosis in Mice**


Gut dysbiosis and disrupted bile acid metabolism, commonly associated with the development and progression of non-alcoholic liver disease (NAFLD), is recapitulated in male mice exposed to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). Previous work demonstrated TCDD increased serum levels of deoxycholic acid, a secondary bile acid solely produced by microbial metabolism exhibiting farnesoid X receptor antagonist activity. The present study demonstrated that TCDD elicited changes in the gut microbiota, male mice (n=3) were orally gavaged with sesame oil vehicle control or 0.3, 3 or 30 µg/kg TCDD every 4 days for 28 days. Shotgun metagenomic sequencing of the cecal contents identified shifts in taxa and microbial metabolic pathways. At the phylum level, dose-dependent decreases in the Bacteroidetes/Firmicutes ratios were consistent with the antimicrobial effects of TCDD-induced bile acids. Many species including *Turbibacter sanguinis* and *Lactobacillus reuteri* which contain genes associated with bile salt hydrolases also exhibited dose-dependent increases. In addition, metagenomic functional analysis revealed TCDD enriched for species contributing to fermentation pathways associated with metabolites known to impact NAFLD progression (i.e. acetate and alcohol). Our results suggest TCDD dose-dependently increased species capable of bile acid metabolism in response to altered bile acid levels and to metabolic pathways that produced microbial products relevant to NAFLD progression. RRF is supported by the National Institute of Environmental Health Sciences (NIH) Multidisciplinary Training in Environmental Toxicology (NIAMS EHS 7T32ES007255). This work was funded by the NIEHS Superfund Research Program (NIEHS SRP P42ES04911), and the NIEHS R01ES029541.

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**2166 Metabolic Reprogramming following Transsulfuration Pathway Inhibition by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to Support Glutathione Biosynthesis in Mice**


Activation of the aryl hydrocarbon receptor (AhR) by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induces steatosis and its progression to steatohepatitis with fibrosis. Oxidative stress is a key contributor to the etiology of these pathologies that is counteracted by antioxidant defenses such as glutathione. To examine the effect of TCDD on transsulfuration pathway and glutathione metabolism, C57BL/6 mice were orally gavaged with TCDD (0.01-30 µg/kg) every 4 days for 28 days. It was shown that TCDD dose-dependently repressed the transsulfuration pathway, specifically cystathionine beta-synthase (CBS) and cystathionine gamma-lyase (CTH), by 2.6- and 2.1-fold at the mRNA level, and 4.1- and 8.7-fold at the protein level, respectively. Accordingly, cystathionine levels were decreased at 1, 3 and 10 µg/kg TCDD but recovered at 30 µg/kg TCDD, while cysteine levels were dose-dependently increased consistent with the 924.5-fold induction of SLC7A11 which encodes for the cystine/ glutamate transporter. Next to cysteine synthesis, CBS and CTH contribute to liver production of H$_2$S. TCDD-induced dose-dependent decrease in expression of these genes was coinciding with dose-dependent decrease in H$_2$S production. In addition, the glutamate-cysteine ligase catalytic subunit (GCLC) and glutathione synthetase (GSS) gene expression were induced 4.9- and 3.8-fold, respectively. The GSSH/GSSG ratio decreased from 80 to 4 at 30 µg/kg TCDD consistent with increased levels of oxidative stress and protein glutathionylation. These results suggest cysteine transport was induced in response to the inhibition of the transsulfuration pathway by TCDD to support glutathione biosynthesis in an oxidative environment. Funded by Superfund Research Program P42ES04911, NIEHS grant (R01ES029541) and the NIEHS R01ES029541.

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**2167 Von Willebrand Factor Exerts Hepatoprotective Effects in Acute but Not Chronic Cholestatic Liver Injury in Mice**

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Acute and chronic liver disease are associated with substantial alterations in the blood clotting system, including elevated levels of the platelet-adhesive protein von Willebrand factor (VWF). We have previously shown that VWF contributes to liver fibrosis in an experimental setting of chemical-induced...
liver injury. Interestingly, in the setting of biliary injury and fibrosis induced by the xenobiotic alpha-naphthylisothiocyanate (ANIT), several studies have documented an anti-fibrotic role for some components of the blood clotting system, including platelets. However, the role of the platelet-adhesive protein VWF in experimental cholestasis has not been examined. We tested the hypothesis that VWF exerts hepatoprotective effects in cholestatic liver disease. Wild-type (WT) and VWF-deficient (VWF⁻/⁻) mice were challenged with either a single dose of ANIT (60mg/kg) or chronic exposure through diet containing 0.05% ANIT. Control mice received vehicle (corn oil) or regular diet. The impact of VWF deficiency on acute biliary injury and liver fibrosis was determined. Acute ANIT-induced liver injury was associated with increased VWF plasma antigen and activity levels. In both wild-type and VWF⁻/⁻ mice, platelets accumulated within the necrotic lesions, and in wild-type mice, co-localized with VWF. Interestingly, and in contrast to our expectation, platelet accumulation was increased in necrotic lesions in ANIT-challenged VWF⁻/⁻ mice. VWF deficiency exacerbated acute ANIT-induced biliary injury as evidenced by increased ALT and hepatocellular necrosis. In concert with liver injury, accumulation of VWF was observed in hepatic platelet accumulations, suggesting that VWF deficiency had no impact on biliary fibrosis induced by chronic ANIT exposure. Collectively, the results indicate that VWF plays dichotomous roles in experimental acute and chronic ANIT-induced liver injury.

2168 Dioxin Reduces Cobalamin Levels and Inhibits Methylmalonyl-CoA Mutase Redirecting Propionyl-CoA to the β-Oxidation-Like Pathway Resulting in Toxic Intermediate Acry-CoA Accumulation

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a persistent environmental contaminant and the prototypical ligand for the aryl hydrocarbon receptor (AhR). AhR mediates the effects of TCDD and related compounds, including the reprogramming of intermediate metabolism. Untargeted metabolomics analysis of hepatic extracts prepared from mice orally gavaged with TCDD every 4 days for 28 days identified the dose-dependent induction of acryl-CoA, a highly reactive toxic intermediate produced in the cobalamin (Cbl)-independent β-oxidation-like metabolism of propionyl-CoA. Acryl-CoA is a biomarker of inborn errors of metabolism associated with propionic- and methylmalonic acidemia due to defects in Cbl-dependent methylmalonyl mutase (MUT) and/or Cbl deficiency. Although both the canonical Cbl-dependent carboxylase and the alternate Cbl-independent β-oxidation-like pathways were affected, gene repression occurred only at 30 µg/kg TCDD while acryl-CoA levels increased at 3 µg/kg. In contrast, TCDD decreased serum Cbl and hepatic cobalt levels at 3 µg/kg TCDD consistent with the dose-dependent increase in acryl-CoA levels. TCDD elicited negligible effects on the expression of genes associated with Cbl absorption, transport, trafficking and derivation to 5-deoxyadenosylcobalamin (AdoCbl), the required MUT cofactor. In addition, TCDD induced Acd1 which converts cis-aconitate to itaconate in macrophages. Iaconate can be converted to itaconyl-CoA, a MUT suicide inactivator that forms an adduct with AdoCbl. Collectively, these data suggest TCDD reduced Cbl levels inhibit MUT activity due to Cbl depletion causing propionyl-CoA metabolism to be redirected to the alternate Cbl-independent β-oxidation-like pathway resulting in acryl-CoA accumulation. RRF is supported by the NIHES Multidisciplinary Training in Environmental Toxicology Program (T32ES007255). This project was funded by the Superfund Research Program (P42ES004911).

2169 Inhibition of Mitochondrial Fatty Acid Oxidation Reduces Liver Injury but Increases Mortality in a Standard Mouse Model of Acetaminophen Overdose

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Acetaminophen (APAP) overdose causes severe acute liver injury in humans and mice. In mice, hepatic fat content dramatically increases early after APAP overdose, before hepatocyte death, due to mitochondrial damage and the resulting loss of mitochondrial fatty acid oxidation (FAO). Consistent with that, fatty changes are seen in liver sections from some overdose patients in too. However, the effect of FAO loss on the later liver injury is unknown. We hypothesized that loss of mitochondrial FAO exacerbates APAP injury due to reduced ATP synthesis and accumulation of lipotoxic intermediates upstream of FAO. To test this, mice were fasted overnight and then treated with 300 mg/kg APAP at 0 h, followed by the mitochondrial carnitine palmitoyltransferase inhibitor etomoxir (Eto) or vehicle at 2 h. We then measured liver injury (serum ALT, histology) as well as hepatic triglycerides (TG), long-chain acyl-carnitines (LCACs), total and oxidized glutathione, and JNK activation at 6 h. To our surprise, treatment with Eto reduced liver injury by 74% compared to vehicle control (ALT: 474±66 U/L vs. 1,787±326, respectively). Paradoxically, it also increased mortality from 0% to 33%, likely due to the effect of combined fasting hypoglycemia and FAO inhibition on the brain. Eto also doubled hepatic TG content and LCACs, confirming further inhibition of FAO in the liver over APAP alone. Interestingly, total glutathione, oxidized glutathione, and JNK phosphorylation in the liver were unaltered, indicating that inhibition of mitochondrial FAO does not affect those well-established mechanisms of APAP toxicity. Conclusions: Our data indicate that inhibition of mitochondrial FAO reduces APAP-induced liver injury in a standard mouse model of APAP overdose independent of well-established mechanisms of toxicity. However, it also increases mortality, likely due to overall reduced energy metabolism. In future studies, we will further analyze the metabolic mechanism by which FAO inhibition reduces the liver injury, and determine if it alters survival in fed mice without hypoglycemia.

2170 The Effects of Endurance Training on High Fat Diet-Induced Nonalcoholic Fatty Liver Disease in Male Mice

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Chronic exercise is a therapeutic strategy in the treatment of many chronic diseases in humans, including the prevention and treatment of metabolic diseases such as diabetes mellitus and Non-Alcoholic Fatty Liver disease (NAFLD). Metabolic, cardiorespiratory, and endocrine pathways targeted by chronic endurance exercise have been identified; however, the specific cellular and molecular pathways that are modified by exercise and have preventive or therapeutic relevance to NAFLD remains unresolved. The exercise model used in the current study allows for the quantification of a human-relevant endurance ‘dosage’, and in this study we show hepatic gene expression, and the structure, characteristics, and clinical differences between sedentary and exercised mice after exposure to regular and high fat diet. Chronic exercise modified the transcription of hepatic genes related to liver nuclear receptors, cell growth, fibrosis, inflammation, and oxidative stress, and decreased the amount of fat accumulation in the liver. The comparison between exercised and sedentary high-fat diet groups identified the level of endurance training that was sufficient to convert the steatosis from macrovesicular to microvesicular. On the other hand, the combination of endurance training with change of diet differentially modified the genetic expression of the biomarkers relative to the separate interventions. The combined intervention was sufficient to convert the structure, characteristics, and clinical aspects of the liver from steatoic to healthy. Given our findings, the combination of endurance exercise and change of diet should be considered a therapeutic option for NASH.

2171 Application of Single Nuclei Transcriptomics to Assess the Hepatic Effects of Dioxin

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Characterization of cell specific transcriptional responses to liver toxicants can be lost in the averages of bulk RNA-sequencing (RNA-seq). Emerging single cell/nuclei RNA-seq technologies now allows researchers to evaluate the transcriptomes of individual cell (sub)types from in vivo and in vitro models. To demonstrate the application of this technology for toxicological evaluation of xenobiotics, we performed single nuclei RNA-sequencing (snSeq) on frozen liver samples from male C3HBL/6 mice gavaged with sesame oil vehicle or 30 µg/kg 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) every 4 days for 28 days. A total of 19,907 genes were detected across 16,015 sequenced nuclei from control and TCDD treated livers. Eleven cell (sub)types were identified including distinct pericentral, midzonal, and perportal hepatocyte sub-populations, and reflected the expected cell diversity of the liver. TCDD altered relative proportions of hepatic cell types and their gene expression. For example, macrophages increased from 0.5% to 24.7%, while neutrophils were only present in treated samples, consistent with histological evaluation. The number of differentially expressed genes in each cell type ranged from 122 (cholangiocytes) to 7,625 (midzonal hepatocytes), and partially correlated with the basal expression level of AhR, the canonical molecular target of TCDD. In future studies, we expect functional enrichment analysis of each (sub)type, RAS signaling and related pathways were specifically enriched in the nonparenchymal cells while enrichment of metabolic processes occurred primarily in hepatocytes. Snseq also identified the expansion of a Kupffer cell subtype highly expressing Gpmb, consistent with a population reported in a dietary

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NASH model. Overall, we show how snSeq can be used to decipher the heterogeneity of transcriptional changes and cell population shifts in response to chemical or drug exposure.

**2172** TCDD-Inducible Poly-ADP-Ribose Polymerase (TIPARP) Regulates AhR Biology and Dioxin Toxicity in Rat

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The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that mediates the toxic responses of mammalian, avian, and marine species to 2,3,7,8-tetrachlorodibenzo/p-dioxin (TCDD) and several other polyaromatic environmental toxicants. Reasons for the diversity of toxic responses to TCDD, the cross-species differences in TCDD sensitivity, and the biological roles of AhR are not fully understood. Our lab found that the protein product of an AhR target gene, TCDD-inducible poly-ADP-ribose polymerase (TIPARP), functions to negatively regulate AhR signaling via mono-ADP-ribosylation. TIPARP−/− mice have increased AhR responsiveness and sensitivity to TCDD-induced toxicity, and display a lethal wasting syndrome at lower doses and shorter time points than do TIPARP+/+ mice. It is not known whether the degree of AhR-TIPARP axis conservation between different species may contribute to known species differences in TCDD toxicity. Male and female TIPARP−/− and TIPARP+/+ rats were treated with TCDD and early transcriptional changes, and hepatic RNA-seq analyses are currently underway. These findings demonstrate that in rats, similar to mice, loss of TIPARP increases sensitivity to TCDD toxicity, in the form of increased steatohepatitis, inflammation and an increased sensitivity to lethal wasting syndrome.

**2173** Human Liver Microphysiological System for Studying Acute and Chronic Drug-Induced Liver Toxicity In Vitro


With safety concerns being the major cause for failures in phases 1 and 2 of clinical trials, it is essential the need for more predictive models within drug discovery and development to improve translatability. In particular, models translated in vitro models have several limitations that make testing chronic drug exposure highly challenging. In recent years focus has turned to human in vitro 3D liver models to provide the solution to these challenges. To understand causality and mechanistic aspects of drug-induced liver toxicity in detail, a human liver Microphysiological System (MPS) was used as in vitro model. Highly functional 3D liver microtissues were maintained under flow perfusion using the PhysioMimix™ MPS for up to 3 weeks. Using the MPS platform a broad spectrum of functional liver-specific endpoints were analysed (inc. clinical biomarkers), highlighting the MPS’ superiority to standard in vitro models. Acute (48 hrs) and chronic exposure (192 hrs) to two antidiabetic thiazolidinediones induced the investigated (troglitazone - known DILI caused by reactive metabolites) (pioglitazone - low DILI concern). Functional liver-specific endpoints were analysed from the culture medium (LDH, albumin, urea, ALT) and liver microtissues (ATP, CYP3A4), to create a distinct mechanistic "signature of hepatotoxicity". Troglitazone caused acute, cmax driven, toxicity that was detected by a number of endpoints (IC50 (pH 7.4) = 109.90 μM, IC50 (pH 5.0) = 69.09 μM and IC50_Alt/min = 94.46 μM). Using the cellular endpoints of ATP content and CYP450 activity, both acute and chronic toxicity was detected with similar IC50 values. Following exposure to pioglitazone some mild chronic toxicity was detected but again only for a subset of endpoints, demonstrating the important of understanding the full signature of hepatotoxicity. Data was generated from independent studies conducted on the same primary human hepatocytes donor which demonstrated high levels of inter- and intra-study reproducibility. Overall demonstrate the liver MPS in vitro model is ideally suited to exploring the molecular mechanisms that underlie DILI and its association with hepatic toxicity. It will also be a highly useful tool for analysing the toxicity profiles of novel compounds and how they may behave in diverse patient subsets.

**2174** Lipid Loading in Micropatterned Primary Hepatocyte and Kupffer Cell Co-culture: NAFLD Disease Modeling for Drug Toxicity Screening


Non-alcoholic fatty liver disease (NAFLD) is a disorder characterized by hepatic steatosis which can advance to non-alcoholic steatohepatitis (NASH). NAFLD and NASH can increase sensitivity to drug induced liver disease (DILI) via changes in drug metabolizing enzymes (DME) and transporters, inflammation, and oxidative stress. Some drugs are also known to cause or worsen NAFLD and NASH. Enhanced DILI in NAFLD often results from chronic drug treatment, making in vitro screening for these outcomes difficult. Here, we show lipid loading and lipid induced toxicity capabilities in HEPATOMUNE® - a long-term micropatterned hepatocyte co-culture model that retains physiological hepatic function for at least 28 days. Diets high in fat and/or carbohydrates are linked to the development of NAFLD. To mimic these conditions, media supplemented with 0.5mM free-fatty-acids (FFA) consisting of a 1:2 ratio of oleic and palmitic acids or high glucose (10g/ L) and fructose (1g/L) (HGF) was used, with lipid assessments after 4 and 10 days of culture. lipids were labelled with BODIPY Solaris and quantified using a Perkin Elmer Operetta CLS high content imager (HCl). Both FFA and HGF resulted in lipid loading, which was more pronounced with the FFA treatment (15.7- and 6.3-fold increase from NT, respectively). In parallel, cells were treated with FFA or HGF for 7 days and cultures were returned to control media for an additional 3-days to assess reversal of lipid loading. While FFA treated cells unloaded lipid, HGF treated hepatocytes retained their lipid stores. In all conditions, lipid loading could be prevented or reversed in a concentration dependent manner using three different ACC1/2 inhibitors (Fisocostat, MK-4074, and PF-05175157). Inflammation is also known to contribute to NAFLD/DILI development and DILI. This aspect was integrated by adding Kupffer cells (HEPATOMUNE®). Under HGF and FFA loading HEPATOMUNE cultures showed a 50% reduction in viability when given a strong inflammatory signal (50ng/mL LPS) - as compared to HEPATOMUNE cultures with no LPS and steatosis stimuli, or those with no Kupffer cells. HEPATOMUNE model thus can serve as an easy-to-use, in vitro platform capable of examining the interplay between FFA, HGF, and immune cell activation on hepatic steatosis and hepatotoxicity- making it a useful platform to study NAFLD related drug toxicity and screen therapeutic modalities.

**2175** High-Throughput NASH Drug Efficacy Testing Using a Scalable Human 3D In Vitro Discovery Platform


Non-alcoholic steatohepatitis (NASH) is a severe, progressive disease characterized by fat accumulation, inflammation, and fibrosis of the liver. Despite the severity and increasing prevalence of this disease, no approved treatments are as yet available. Ongoing drug discovery and development has proven challenging due to the lack of suitable in vivo and in vitro preclinical models that recapitulate all aspects of NASH. The aim of this study was to develop a scalable, high-throughput-screening platform for drug efficacy testing, based on a human-cell-based 3D in vitro NASH model, to enable predictive and efficient screening of NASH compounds and combination therapies. We produced scaffold-free 3D microtissue co-cultures of primary human hepatocytes, Kupffer cells, liver endothelial cells and hepatic stellate cells, then induced NASH using a cocktail of lipotoxic and inflammatory stimuli (free fatty acids and LPS) in media containing high levels of sugar and insulin. Compared to untreated controls, disease-induced models displayed key pathophysiological features of NASH after 10 days of treatment: 1) increase of intracellular triglyceride content as an indicator of fat accumulation; 2) secretion of inflammatory cytokines/chemokines, such as IL-6, MIP-1α, TNF-α, IL-10, MCP-1 and IL-8; and 3) increased fibril collagens deposition and secretion of procollagen type I and III peptides. Whole transcriptome analysis of NASH-treated models versus control revealed activation of pathways and differentiation of regulatory genes associated with key processes in NASH, such as lipid metabolism, inflammation, and fibrosis induction. Treatment with anti-TGF-β antibody and ALK5i (TGFβRI inhibitor) concentration dependently decreased secretion of pro-collagen type I/III. Decreased deposition of fibrosis based on quantification of Sirius-Red staining (PharmaNest) was observed in microtis...
sues treated with anti-TGF-β antibody and ALK5i. Importantly, treatment with anti-NASH drug candidates (Selonsertib and Firisocostat) affected biochemical endpoints indicative of disease progression, and results were to a large extent in line with clinical observations. In summary, this high-throughput screening platform is a promising research tool for rapid evaluation and selection of most effective novel NASH drug candidates to advance in the NASH development pipeline.

**2176 Analyses of the Effects of an Environmentally Relevant Organophosphate Ester Mixture in the HepG2 Cell Line Using High Content Imaging**

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Organophosphate esters (OPEs) are commonly used in commercial products as flame retardants or plasticizers. However, OPEs are not chemically bound to these products and leach out into the environment; OPEs have been detected in indoor air, household dust, drinking water, sediment and biota. Humans are exposed to OPEs through dermal contact, ingestion and inhalation. House dust is a major source of exposures. House dust samples collected in Canada between 2007-2010 were found to contain 13 OPEs. Studies have shown that some of these OPEs may bioaccumulate in the liver and cause adverse health effects. Here we have tested the hypothesis that exposure to the mixture of OPEs found in Canadian house dust will have adverse effects on HepG2 human liver cells. HepG2 cells were exposed to vehicle control (DMSO) or a series of OPE mixture dilutions for 48 hrs. OPE mixture dilutions include 1/1,000,000X; 1/300,000X; 1/100,000X; 1/75,000X; 1/60,000X; 1/45,000X; 1/30,000X; 1/10,000X; 1/3,000X, where 1X corresponds to 5.005 g of the OPE mixture found in Canadian house dust. Cytotoxic, mitochondrial activity, oxidative stress levels, lysosomal intensities and lipid droplet areas were screened using a high content imaging system with fluorescent staining. Exposure to OPE mixture dilutions of 1/10,000X and 1/3,000X were cytotoxic, hence these two dilutions were excluded from further analyses. Mitochondrial labelling and oxidative stress were not affected by OPE exposure. However, there was a significant increase in lysosomal intensity at all dilutions tested. There was also a dilution-dependent increase in lipid droplet areas after exposure to OPE dilutions of 1/60,000X, 1/45,000X and 1/30,000X. Therefore, these results suggest that in vitro exposure to an environmentally relevant OPE mixture may have adverse effects on the liver. Supported by OHRR, CRRD and McGill University.

**2177 Lack of Direct Detectable Facts of Silver and Silver Nanoparticles on Mitochondria in Mouse Hepatocytes**

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Silver nanoparticles (AgNPs) are well-proven antimicrobial nanomaterial, which hold great promise for a wide range of biological applications. Despite the promising advantages, significant human exposure to AgNPs has raised concerns over potential human health and environmental hazards. While multiple studies have investigated the toxic effects of various AgNPs in different model systems, the underlying mechanism is still elusive until recently. The endosymbiotic theory holds that mitochondria are the descendants of proteobacteria that originally lived inside ancestral eukaryotic cells. To determine whether mitochondria are the primary target of Ag toxicity, we explored the possible relationship between mitochondrial activity and the cytotoxicity of AgNPs with different coating and sizes, including silver ions, in AML12 cells cultured in glucose and galactose-based media. AML12 cells were found to rely mostly on oxidative phosphorylation (OXPHOS) to produce their ATP by substituting glucose for galactose in the cell culture medium. Therefore, they became more sensitive to mitochondrial toxins than cells grown on glucose medium. However, to our surprise, we found that 15 nm AgNPs, 6 nm Ga-AgNPs, and AgNO3 failed to cause greater toxicity to AML12 cells grown on galactose than glucose at indicated concentrations. Meanwhile, overall levels of colocalization between 15 nm AgNPs and mitochondria were relatively low, and once again, no difference was found between glucose and galactose-based cells. By quantifying Ag content at sub-cellular levels, we verified large amounts of Ag localized at cytosol fractions instead of mitochondrial fractions, consistent with the fluorescent images. Furthermore, our data illustrated that, under sub-toxic conditions, the effects of AgNPs and AgNO3 on mitochondrial function were mild; this result was inconsistent with mitochondrial toxicity causing cell death. In summary, our results suggest that silver exposure (AgNPs and AgNO3) do not specifically target mitochondria, nor do we have direct evidence that mitochondrial dysfunction is the primary cause of cell death after Ag exposure.

**2178 In Vitro Antimicrobial and Cytotoxic Investigation of Chitosan Nanoparticles Extracted from Black Soldier Fly (Hermetia Illucens) Waste Material**

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Chitosan is a biopolymer extracted from chitin by process of deacetylation. Insect chitosan is non-allergenic and with increased investment could replace the traditional shrimp chitosan sources. Black soldier fly (BSF) could offer this alternative since its farming has attracted increased global attention with its attendant waste problems. This study is aimed at evaluating the effects of BSF chitosan nanoparticles (BSF_NPs) synthesized by electrospraying technique on microbial cultures. The study further examined the cytotoxicity of the BSF_NPs on human skin cell line. BSF chitosan was obtained by chemical extraction technique. The chitosan was purified and electrosprayed into nanoparticles (BSF_NPs). The BSF_NPs were characterized using Fourier transform infrared spectroscopy (FTIR), high resolution scanning electron microscopy (HRSEM) and zeta sizer. An antimicrobial investigation was carried out using the time-kill technique on Gram-positive E. faecalis (ATCC 29212) or Gram-negative S. mutans (ATCC 25175). The cytotoxicity of 1.0 mg/mL of the BSF_NPs was carried out using the JR human skin fibroblast cell lines following the MTT assay technique. The result of the characterization of the BSF_NPs showed an average hydrodynamic particle size of 289±98 nm resulting from the dynamic light scattering technique. The BSF_NPs displayed similar spectral features as the bulk chitosan sample and a high positive zeta potential value of 54.7±2.96 mV with a polydispersity index of 0.16. The BSF_NPs were effective and eradicated microbial cells of E. faecalis or S. mutans within 30 minutes of contact. The cytotoxic investigation showed that the BSF_NPs did not inhibit the proliferation of the skin cells after 24 h, rather the cells were viable and increased by 5% compared to the control samples. Therefore, the BSF_NPs used in this study eliminated microbial cells of E. faecalis or S. mutans but promoted the growth of the human skin fibroblast cells. This points to the ability of the BSF_NPs to sterilize microbial environment while stimulating the healing of human skin cells when exposed to it. This study, therefore, lays credence to the use of chitosan nanoparticles from BSF in biomedical applications such as wound dressing, targeted nano-systems for cancer treatment, and so on since they are non-allergic to humans.

**2179 Effects of Carbon Nanodots Derived from Ethylenediamine and Citric Acid on Oxidized-LDL-Induced Inflammation in Human Endothelial Cells**

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Cardiovascular disease is the leading cause of death in the United States. Oxidized-LDL (Ox-LDL) is a known biomarker of inflammation, and Ox-LDL can induce inflammatory gene expression and monocyte extravasation, leading to the development of atherosclerosis. Carbon nanodots (CNDs) are a new class of nanoparticles that are used in drug delivery, diagnostics, and bioimaging. This study examines the role of CNDs in mediating Ox-LDL-induced inflammation in human microvascular endothelial cells (HMEC-1). Our results demonstrate that CNDs can reduce Ox-LDL induced monocyte adhesion in HMEC-1s, which indicates that they have anti-inflammatory effects. In the presence of CNDs, the relative gene expression of the cytokine interleukin-8 (IL-8) was reduced, which implies their action in mediating monocyte recruitment to the site of inflammation. Overproduction of reactive oxygen species can induce oxidative stress, endothelial dysfunction, inflammation. The study of CND's role by electron paramagnetic resonance (EPR) spectroscopy shows that CND can directly scavenge superoxide and hydroxyl radicals. This result suggests that the anti-inflammatory effect of CND is probably due to its direct elimination of ROS. In addition, CND was found to ameliorate the cytotoxicity caused by Ox-LDL in HMEC-1s. These results demonstrate the potential of CNDs to reduce Ox-LDL-induced inflammation and cytotoxicity in HMEC-1s, which implies their use in developing new therapies for cardiovascular diseases.
We investigated the cytotoxic effects of uncoated and polyethylene glycol (PEG)-coated gold nanoparticles (AuNPs) on human kidney cells. Both uncoated and PEG-coated AuNPs were synthesized following standard protocols and characterized by transmission electron microscopy (TEM). Their size and distribution were measured by dynamic light scattering (DLS), and the zeta potential was determined using Zeta Sizer Nano ZS analyzer. Cytotoxicity was measured by an MTT assay and trypan blue exclusion test, and oxidative stress was assessed by quantifying the levels of reactive oxygen species (ROS), reduced glutathione (GSH), and mitochondria membrane potential (MMP). Uncoated AuNPs significantly reduced cell viability, increased ROS, decreased GSH, and depolarized the MMP under concentrations-dependent manner. PEG-coated AuNPs displayed a lower toxicity, and did not produce any significant increase in ROS or significant decrease in GSH and MMP. Hence, oxidative stress plays a role in AuNPs-induced cytotoxicity in HK2 cells. Also, PEG-coated AuNP are relatively less cytotoxic than uncoated AuNPs and hence, may have potential applications in nanomedicine.
2184 Oxidative Stress-Induced Junction Complex Changes in Human Intestinal Epithelial Cells after Dietary Nanoparticles Exposure
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The concerns about the health risks of ingested nanoparticles (NPs) are on the rise due to their increasing applications in food additives, nutraceuticals and packaging materials. In this study, the most widely used NPs in food industry such as food grade silicon dioxide (SiO₂), titanium dioxide (TiO₂) and silver (Ag) along with their non-food grade variations and bulk counterparts were selected as the library of particles. These particles were characterized for their size, agglomeration, surface charge and were exposed to human intestinal epithelial cell models including Caco-2 and HIEC-6 cells to characterize the responses at molecular, cellular and intracellular level after treatment. The study demonstrated that upon cellular exposure, AgNPs can trigger the orchestra of changes in oxidative stress, Ca²⁺ flux and mitochondria function, which can further impairment of cellular junction complex function. The ultrastructure was assessed via transmission electron microscopy. Differential dose-response patterns were seen in the assessments as an increase in multispecific bodies formation and a decrease in the number of mitochondria per cell. The ultrastructural alterations were not statistically significant and indicate that there were no cellular structure changes over each dose. The results in this study indicate that there is no endocrine disrupting potential induced after CNC exposure. This information will shed light on sex-specific exposure responses as well as help inform regulators and risk assessors of the potential effects of CNC use in new products. Additionally, we hope that the future of nanomaterial production will include endocrine disrupting potential as an end of lifecycle test to uphold the highest of safety testing. These studies aid in the advancement of safety measures when producing and utilizing nascent renewable resources.

2185 Effects of Multiwalled Carbon Nanotubes on Immune Cells in a Hepatocyte Co-culture Setup In Vitro
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Exposure to Multi-walled carbon nanotubes (MWCNT) can occur through drug carriers and medical devices. MWCNTs that enter the blood circulation end up in the liver. These nanoparticles can produce inflammation and toxicity or suppress immune function. Although the liver is primarily involved in metabolic processes, it cannot metabolize nanoparticles. The effects of these nanoparticles alone or in combination with other hepatotoxins such as acetaminophen on the liver is not clearly understood. High mobility group box-1 (HMGB1) is considered a mechanistic marker for liver injury. We have reported increased HMGB1 expression in a hepatocyte cell line (HC-04) due to MWCNT and APAP co-exposure. In the current study we modeled liver by co-culturing hepatocytes (HC-04) with macrophages (differentiated THP1) and evaluated whether macrophages enhance the sensitization of hepatocytes (HC-04) to MWCNT- and APAP-induced cytotoxicity. We also evaluated the uptake of carbon nanoparticles by imaging using transmission electron microscopy. HC-04 and THP1 cells co-cultures were exposed to 1 µg/mL MWCNT for 72h and subsequently treated with 15 mM APAP for 24h. At the end of the study, cytotoxicity was quantified by measuring the lactate dehydrogenase (LDH) leakage into the media. mRNA expression was measured by quantitative real-time PCR. Results show that percent LDH leakage decreased in co-cultures receiving APAP and MWCNT, suggesting decreased toxicity. On the contrary, mRNA expression of inflammatory cytokines (IL-1β, TNFα) increased in the co-cultures receiving co-treatment suggesting immune cell activation. This is consistent with the observation that nanoparticle uptake by macrophages is more pronounced than hepatocytes. The consequences of uptake and the mechanism of immune cell activation due to co-exposure warrants further investigation.

2186 A Study of Renewable Nanomaterials: Cellulose Nanocrystals Are Not Found to Have Endocrine-Disrupting Potential
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Cellulose is a renewable and generally regarded as safe (GRAS) resource used as an additive in consumer products including paper and mill, food packaging, and cosmetics to name a few. Interest in the use of engineered cellulose structures, cellulose nanocrystals (CNC), is rapidly increasing as more studies uncover innovative uses for this diverse material. Traditionally when there is an emergence of a new nanomaterial in consumer products and industrial processes, there is the potential to pose hazards to the health and wellness of consumers and operational workers. Previous studies have shown that inhaling CNCs can cause an inflammatory response where sex influences the severity of adverse outcomes. In this study, a simulated CNC exposure was performed to probe key molecular biosignatures in the endocrine disruption stereogenic pathway. Primary human adrenal gland cells were used to monitor the endocrine disruption potential due to their use as the ‘gold standard’ of endocrine disruption testing with chemicals. The expression level of eleven different stereogenic genes and 5 inflammatory genes were measured using quantitative real-time polymerase chain reaction (qRT-PCR). Several genes in the stereogenic pathway expressed an increase in the fold change in the 50mg/m³ overload concentration, however no endocrine disruption potential was seen at lower concentrations. Additionally, ultrastructure was assessed via transmission electron microscopy. Differential dose-response patterns were seen in the assessments as an increase in multispecific bodies formation and a decrease in the number of mitochondria per cell. The ultrastructural alterations were not statistically significant and indicate that there were no cellular structure changes over each dose. The results in this study indicate that there is no endocrine disrupting potential induced after CNC exposure. This information will shed light on sex-specific exposure responses as well as help inform regulators and risk assessors of the potential effects of CNC use in new products. Additionally, we hope that the future of nanomaterial production will include endocrine disrupting potential as an end of lifecycle test to uphold the highest of safety testing. These studies aid in the advancement of safety measures when producing and utilizing nascent renewable resources.

2187 A Self-Emulsifying Drug Delivery Platform for Enhanced Cisplatin Activity in Triple-Negative Breast Cancer
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Cisplatin, a platinum-based antineoplastic drug is widely used in the treatment of several cancers. Tumor resistance, as variable resistance to cisplatin, response to cisplatin in the triple-negative breast cancer (TNBC) subtype limits its clinical use and long-term efficacy. This study proposes a formulation-based approach to improving therapeutic outcomes with cisplatin using a self-emulsifying drug delivery system (SEDDS). Cisplatin-SEDDS formulations (Cis-SEDDS) were prepared and characterized for particle size, polydispersity, and zeta potential. SEDDS were optimized using pseudo-ternary phase diagrams to facilitate self-emulsification of LabraPar PG, Labrasol, and Gelucire 44/14, without and with with Compritol (Cis-SEDDS 1) and (Cis-SEDDS 2) respectively in water. Cellular uptake studies using tripleplex stained were co-cultured with human hepatocytes (HC-04) and auto-induced loaded SEDDS, Lysotribe, THP1, and MDA-MB-231 cells. Cytotoxicity was investigated using resazurin reduction assay and results presented as average cell viability with SEM. Induction of apoptosis was investigated using acridine orange-ethidium bromide (AO-EB) assay. Stable emulsification was achieved for both formulation types with up to 60% (w/v) oil phase for at least 10 days. SEDDS ranged in size, PDI, and surface charge from 121 - 221 nm, 0.153 - 0.207, and -6.87 - -7.29 mV, respectively. The Cis-SEDDS formulation showed great internalization of doxorubicin ensuring good cellular uptake. Average cell viability was decreased across dilutions by Cis-SEDDS 1 (94%) and Cis-SEDDS 2 (90%), compared to cisplatin solution (99%). At 10 µM, significant inhibition of cell viability was observed for Cis-SEDDS 2 (83%) compared to Cis-SEDDS 1 (94%) and cisplatin solution (92%). Compared control and cisplatin solution, Cis-SEDDS were relatively non-toxic to MDCCK cells while inducing apoptosis in MDA-MB-231 cells. Cis-SEDDS 2 significantly inhibited MDA-MB-468 cell viability, potentially through the sustained-release of cisplatin by Compritol. A SEDDS formulation approach may be useful in enhancing the anticancer effects of chemotherapeutics such as cisplatin and rescuing from tumor resistance.

2188 Declines in Serum Levels of Apolipoproteins during the Development of Peritoneal Mesothelioma by Multiwalled Carbon Nanotube in Rats
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A rat model of the induction of mesotheliomas by an intraperitoneal injection of multiwalled carbon nanotube (MWCNT) is useful to understand mechanisms underlying fibrous particles-associated carcinogenesis. Although there is a need to efficiently evaluate chronic toxicities of numerous nanomaterials, promising biomarkers were rarely reported, in particular, for mesotheliomas. This study aimed to search for serum proteins differentially expressed during the development of mesothelioma by MWCNT. A total of 12 male F344
rats were divided equally into 2 groups, intratracheally injected vehicle or MWNT-7 at 1 mg/kg body weight (BW) (a MWNT dose inducing peri toneal mesothelioma in all treated rats within a year) and euthanized to collect blood samples at the end of week 32. Serum samples were then prepared and processed for the proteomic analysis. A 2-dimensional gel electrophoresis revealed a total of 259 protein spots with significantly altered intensities, 13 of which were identified by a nano LC-MS/MS. Among such proteins, the declined level of allopurinol A-V was validated in 3 different experiments: i) a time-course study in which the rats were dosed with 1 mg/kg BW of MWNT-7 and sequentially necropsied for 32 weeks, ii) a 2-year carcinogenicity test for MWNT-7 with doses of 0.05 or 1 mg/kg BW, and iii) a 1-year comparative analysis for 2 different types of MWNTs, SD-1, a thick and long fiber (car cinogenic), and SD-2, a thin and tangled fiber (non carcinogenic) with doses of 1 mg/kg BW. As for all animals, serosal tissues of the coelomic organs were histopathologically evaluated and serum levels of a set of lipoprotein-related molecules were biochemically analyzed. As a result, serum levels of apolipopro tein A1 and B weight and a ratio of HDL to LDL were time-dependently decreased during the development of mesothelioma, and they were inversely correlated with the severity of the tumor. This study provides potent biomarkers in association with the induction of mesothelioma by MWNT and a new indication of the dysregulation of the lipid homeostasis in mesotheliomagenesis. Funded by Health and Labor Sciences Research Grants (H30-Kagaku-Shitetsu-004) from the MHLW, Japan.

**2189** Pulmonary Effects of Fe3O4-PEG-PLGA Nanoparticles in Human Bronchial Epithelial Cells and in Wild-Type and Nrf2 Knockout Mice following Pharyngeal Aspiration


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Fe3O4-PEG-PLGA nanoparticles (NPs) are promising bionanomaterials due to their potential application in medical areas. The present study investigated pulmonary effects of exposure to Fe3O4-PEG-PLGA NPs and role of Nrf2 in them. Male C57BL/6J/Jcl wild-type (WT) and Nrf2 KO mice were given a single dose of 0, 10, or 30 µg Fe3O4-PEG-PLGA NPs by pharyngeal aspiration. To confirm effects on human cells, air-liquid interface cultures of the bronchial epithelial cell line Calu-3 were treated with equivalent doses (20 and 60 µg/cm²). 14 days after exposure, mice were euthanized and bronchoalveolar lavage fluid (BALF) was collected. Total protein (TP), total and differential cell counts in BALF were examined. Lungs were sectioned and stained with Prussian Blue. Gene expression of selected proinflammatory cytokines in mice lung and human cell cultures were checked by qPCR. Cytotoxicity, barrier function and cytokine release were also evaluated in Calu-3 cultures. Exposure to 30 µg Fe3O4-PEG-PLGA NPs increased total cells and macrophages in WT mice. TP, lymphocytes, neutrophils and eosinophils in Nrf2 KO mice, and basophils in both genotypes. Prussian Blue staining of the lung showed internalization of Fe3O4-PEG-PLGA NPs by macrophages in both genotypes exposed to 10 or 30 µg of Fe3O4-PEG-PLGA NPs. Exposure to Fe3O4-PEG-PLGA NPs increased dose-dependently pulmonary expression of TNFα and IL-1β mRNA in Nrf2 KO mice, while its expression was decreased in WT mice. Interleukins (IL-6, CXCL1, CXCL2), TNFα (IL-1β, IL-13), and tissue remodeling (TIMP1, MMP9) cytokines. High dose Cloisite 93A and 1% Cloisite 93A PP also elicited M1Pα, MDC, MMP9, CXCL2, IL1α release on day 7 and elevated M1Pα, IL5, and IL6 on day 28 compared to other treatments. In summary, Nrf presence and particle source along the ONC lifecycle influenced leukocyte airway, interstitial, and inflammatory marker responses.

**2190** Pulmonary Inflammation Response Comparison of Nano-Clay to Machined Dusts of Nano-Clay-Enabled Composite

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One of the concerns with inhalation of nanomaterials is the possibility that due to their small size, nanomaterials may translocate to, and cause adverse effects in tissues and organs beyond the lungs. Carbon black is an industrially produced particulate form of elemental carbon, and is often used in particle toxicology investigations as a reference nanomaterial. Although the primary particles of carbon black may be in the nanosize range, carbon black invariably exists outside the reactor in which they are formed only in the form of aggregates which are collections of fused primary particles, and agglomerates which are collections of weakly bound aggregates. To understand the fate of carbon black in the body, a high surface area form of carbon black was radiolabeled with the gamma tracer beryllium-7 by proton beam irradiation. A purification sequence was performed to remove any soluble Be7 by successive washing with ethanol solution, slight hydrochloric acid and artificial lung fluid. The final Be7 labeled test item was collected on a filter. Approx. 0.3 mg of carbon black was intratracheally instilled into rat lungs, an amount which was not expected to cause rat lung overload. The urines/feces excretion, CD4+ T cells (day 1) while NP exposed animals showed elevated interstitial B cells, CD4+ T cells, and CD8+ T cells dose-dependently in particle-exposed mice. Neutrophils infiltrates persisted up to day 28 in Cloisite 93A-exposed mice. Cloisite 93A caused elevated interstitial CD4+ T cells (day 1) while NP exposed animals showed elevated interstitial B cells. Particle exposure caused transient increases in cDC2s, pDCs, interstitial macrophages, and activated (CD86+) monocytes. Cloisite 93A induced elevated CD11b+ DCs (day 1 and 7) and CD86+ CD103+ DCs (day 1 and 28). Irrespective of ONC or particle, hyper-acute inflammatory cytokine induction was observed, characterized by acute inflammatory (MIP1α, MDC, IL1β, TNFα, IL6, CXCL1, CXCL2), Th2 (IL5, IL13), and tissue remodeling (TIMP1, MMP9) cytokines. High dose Cloisite 93A and 1% Cloisite 93A PP also elicited M1Pα, MDC, MMP9, CXCL2, IL1α release on day 7 and elevated M1Pα, IL5, and IL6 on day 28 compared to other treatments. In summary, ONC presence and particle source along the ONC lifecycle influenced leukocyte airway, interstitial, and inflammatory marker responses.
als affecting human health. GO nanoparticles have been reported to cause toxic effects by dermal, ingestion and inhalation routes of exposure. However, there are limited studies on effects of GO on human health via inhalation, which could be a primary route of exposure in the workplace. The hypothesis to be tested in this study is acute inhalation of 2-D GO results in an acute and transient inflammation of the respiratory tract. Male and female Sprague Dawley rats eight weeks of age were divided into sham control (n=12) or GO exposed groups (n=24). Exposure to aerosolized GO (400nm x 400nm) was for a single 6-hour period in a nose-only inhalation chamber to attain optimal exposure. Particle aerosol was characterized using gravimetric measurements, cascade impactor analysis and transmission electron microscopy. Animals were examined on days 1 and 7 post-exposure. Bronchoalveolar lavage (BAL) was collected from the right lung to assess protein concentration, cell number, viability, and cell differentials. The left lung was inflation-fixed, embedded and sectioned for histopathological analysis. Average GO aerosol mass was 2.28 +/- 0.73 mg/m3. The mass median aerodynamic diameter of the aerosol was 2.37 µm. Morphologically similar and observable for cell differentials, cell number, cell viability or protein concentration between the control and exposed animals for both male and female rats. Hence, the study suggests GO has no acute pulmonary toxicity in rats at the given concentration and duration of exposure following a single day.

**2193** Differentiating Exogenous versus Endogenous Iron Nanoparticle Types in Human Olfactory Bulb with Neurodegeneration: Pollution Particles against Biomineralized Iron

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Aerosol constituents in polluted air contain redox-active iron nanoparticles, and mounting evidence supports their role for tissue interactions that promote neurodegeneration at and beyond the rostral (olfactory bulb (OB)) and caudal (brain stem) point of CNS entry. An increased risk for Alzheimer’s and related dementia has been hypothesized to be associated with airborne nanoparticle transport via neuronal olfactory and trigeminal pathways. We examined autopsied OBS to determine whether inhaled pollution-derived iron reaches the brain via the olfactory nerve. To understand the accumulation of iron nanoparticles in the OB requires to differentiate between the sources of iron coming either from exogenous (aerosol pollution) or endogenous origins (biomineralization) and to quantify the various iron components which is crucial to link exogenous iron to neurodegeneration. Our approach uses analytical high-resolution transmission TEM of OB thin-sections coupled with electron energy loss spectroscopy (EELS) to assess compositions, relative quantities and redox-activities of distinct iron particles and to identify the physical markers fingerprints of individual iron nanoparticles that either translocated to OB regions (exogenous) or precipitated in vivo (endogenous). Analyses were performed using a cryo-stage to stabilize the biological sections. We grouped iron particles with identical characteristics and used digital imaging and spatial statistics to assess their frequency in the OB. Information on exogenous iron nanoparticles has been hypothesized to be associated with airborne nanoparticles transport via neuronal olfactory and trigeminal pathways. We examined autopsied OBs to determine whether inhaled pollution-derived iron reaches the brain via the olfactory nerve. To understand the accumulation of iron nanoparticles in the OB requires to differentiate between the sources of iron coming either from exogenous (aerosol pollution) or endogenous origins (biomineralization) and to quantify the various iron components which is crucial to link exogenous iron to neurodegeneration. Our approach uses analytical high-resolution transmission TEM of OB thin-sections coupled with electron energy loss spectroscopy (EELS) to assess compositions, relative quantities and redox-activities of distinct iron particles and to identify the physical markers fingerprints of individual iron nanoparticles that either translocated to OB regions (exogenous) or precipitated in vivo (endogenous). Analyses were performed using a cryo-stage to stabilize the biological sections. We grouped iron particles with identical characteristics and used digital imaging and spatial statistics to assess their frequency in the OB. Information on exogenous iron nanoparticles has been hypothesized to be associated with airborne nanoparticles transport via neuronal olfactory and trigeminal pathways. We examined autopsied OBs to determine whether inhaled pollution-derived iron reaches the brain via the olfactory nerve. To understand the accumulation of iron nanoparticles in the OB requires to differentiate between the sources of iron coming either from exogenous (aerosol pollution) or endogenous origins (biomineralization) and to quantify the various iron components which is crucial to link exogenous iron to neurodegeneration. Our approach uses analytical high-resolution transmission TEM of OB thin-sections coupled with electron energy loss spectroscopy (EELS) to assess compositions, relative quantities and redox-activities of distinct iron particles and to identify the physical markers fingerprints of individual iron nanoparticles that either translocated to OB regions (exogenous) or precipitated in vivo (endogenous). Analyses were performed using a cryo-stage to stabilize the biological sections. We grouped iron particles with identical characteristics and used digital imaging and spatial statistics to assess their frequency in the OB. Information on exogenous iron nanoparticles has been hypothesized to be associated with airborne nanoparticles transport via neuronal olfactory and trigeminal pathways.

**2195** Thin-Tangled Multivalved Carbon Nanotubes Are Carcinogenic to the Rat Lung after Administration by Intra-tracheal Instillation

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Because of their remarkable physical and chemical characteristics, MWCNTs are used in wide variety of industrial applications. One of the properties of MWCNTs is their very light weight. This makes MWCNTs easily airborne and inhaled. MWCNTs can be generally divided according to their shape into thin tangled and thick straight subtypes. Intraperitoneal injection studies in the rat reported that thick straight types of MWCNTs were carcinogenic while thin tangled types of MWCNTs were not. The purpose of the present study was to investigate the carcinogenic potential of a thick, straight-type MWCNT (MWCNT-A, approximately 150 nm in diameter), and a thin tangled-type MWCNT (MWCNT-B, 7.4 nm in diameter) after administration into the rat lung. Crocidolite asbestos was used as the reference material, and rats administered vehicle were used as the controls. Test materials were administered by intra-Tracheal Intra-Pulmonary Spraying (TIPS) once a week over a 7-week period (8 doses from day 1 to day 50), followed by a 2-year observation period without further treatment. Rats were administered total doses of 0.5 or 1.0 mg/rat MWCNT-A and MWCNT-B or 1.0 mg/rat asbestos. There was no difference in survival between any of the groups. The rats administered MWCNT-A or asbestos did not have a significant increase in bronchiolo-alveolar hyperplasia or tumors in the lung. However, the incidence of bronchiolo-alveolar hyperplasia was 0/20, 1/20, and 2/20 in the vehicle, 0.5 mg MWCNT-B, and 1.0 mg MWCNT-B groups, respectively. Malignant adenocarcinoma combined was 1/19, 5/20, and 7/20 in the vehicle, 0.5 mg MWCNT-B, and 1.0 mg MWCNT-B groups, respectively, and the incidence of adenoma and hyperplasia or tumors in the lung. However, the incidence of bronchiolo-alveolar hyperplasia was 0/20, 1/20, and 2/20 in the vehicle, 0.5 mg MWCNT-B, and 1.0 mg MWCNT-B groups, respectively. Malignant adenocarcinoma combined was 1/19, 5/20, and 7/20 in the vehicle, 0.5 mg MWCNT-B, and 1.0 mg MWCNT-B groups, respectively, and the incidence of adenoma and hyperplasia or tumors in the lung.
mal rat serum, or 10% Met5 rat serum. Components of the Met5 and healthy BC were evaluated via a proteomics approach and differences in specific apolipoproteins were determined. Endothelial cells exposed to NPs with a Met5 BC demonstrated enhanced phosphorylation of AKT (T308) and elevated total and phosphorylated mTOR (S2448) indicative of mTOR complex 1 activation. Markers of apoptosis (cleavage of caspase-3 and cytochrome c levels) were increased only in cells exposed to NPs with a Met5 BC. mTOR signaling induces apoptosis via inhibition of autophagy. Macrophages demonstrated decreased gene expression of LC3, a marker of autophagy, only in cells exposed to NPs with a Met5 BC. Treatment with rapamycin, an inhibitor of mTOR, inhibited changes in LC3 expression. Previously we have demonstrated the NPs interact with scavenger receptors and these interactions were increased by a lipid-rich BC. CD36 was determined to be the primary facilitator of NP internalization and is increasingly exposed due to Met5. A CD36-specific ligand enhanced mTOR and Raptor gene expression similarly to NPs with a Met5 BC. A number of processes down-stream of mTOR (insulin sensitivity and lipogenesis) were also uniquely altered by the Met5 BC. Together these findings demonstrate that in Met5 a unique BC forms that may increase scavenger receptor interactions and thereby enhancing mTOR signaling contributing to exacerbated responses to NP exposures. Funded by NEIHS ES024392.

2197 Exacerbated Acute Inflammatory Response by Nanoparticle Exposures in a Metabolic Syndrome Mouse Model
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Nanoparticles are increasingly utilized in many applications, such as manufacturing processes, electronics, and consumer products, enhancing the potential for human exposures. Epidemiological assessments have established that individuals with underlying chronic diseases are increasingly sensitive to exposures; however, the mechanisms remain unknown. Our previous study demonstrated metabolic syndrome causes enhanced inflammation following nanoparticle exposure compared to healthy due to reductions in lipid mediators of inflammatory resolution. Our current study hypothesizes that specific resolution mediators are impaired in metabolic syndrome exacerbating inflammatory responses following exposure. To evaluate this hypothesis, healthy and metabolic syndrome were injected with saline (control), 14-HDHA, 17-HDHA, or 18-HEPE at 1 μg. After supplementation, mice were exposed to saline or 50μg of 20nm silver nanoparticles via oropharyngeal aspiration and necropsied 24 hours post-exposure. Again, metabolic syndrome enhanced pulmonary neutrophil influx, inflammatory gene expression, and bronchoalveolar lavage inflammatory cytokine levels. None of the treatments altered the acute inflammatory response induced in healthy mice in response to silver nanoparticles. Treatment with 14-HDHA and 17-HDHA were determined to reduce exacerbated inflammatory responses in the metabolic syndrome mouse model to levels observed in the exposed healthy model. Additionally, targeted mass spectrometry measured altered pulmonary lipids while Western blots examined metabolic syndrome associated changes in resolution receptors that may impair resolution signaling. This data further demonstrates the role of resolution contributors to increased inflammation associated with metabolic syndrome. Specifically, decreases in specialized pro-resolving mediators of resolvins-1 and resolvins D series produced by 14-HDHA and 17-HDHA appear to mediate exacerbations. A thorough understanding of mechanisms of inflammation is necessary for the development of treatment strategies and interventions. Funded by NEIHS ES024392.

2198 Unified Transcriptomics Data Collection for Toxicogenomic Approaches to Nanosafety

Toxicogenomics has been gaining recognition as a complementary approach to traditional safety assessment of chemical exposures. Omics data can help to unveil the mechanism of action of compounds while facilitating the development of predictive models and other computational strategies for toxicology. Robust models and interpretations, however, require large-scale high-quality data in a uniform format. Engineered nanomaterials (ENMs) are an emerging class of compounds of high industrial interest but still limited information on the safety. Although large amounts of transcriptomics data have been generated to support the safety assessment of ENMs, the data are scattered across public repositories in diverse formats and varying levels of characterization. This harmonizes the demand of FAIRness (Findability, Accessibility, Interoperability and Reusability) of the data, which is highly needed for developing the next generation of integrated methods for safety assessment. In order to unleash the full potential and elevate the level of FAIRness of the publicly available data sources, we manually curated a homogenized transcriptomics data collection of ENM exposures in human, mouse, and rat, representing a range of biological systems and ENMs from a total of 101 publicly available data sets. The collection is supplemented with the physicochemical characteristics of included ENMs, forming the largest unified collection of ready-to-use data for toxicogenomic modeling of ENMs.

2199 Effects of Carbon Nanodots on Cytotoxicity and Tumor Necrosis Factor-Alpha-Induced Pro-Inflammatory Cytokine Expression In Vitro (Endothelial Cells) and In Vivo (C57BL/6)
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Cardiovascular diseases (CVDs) are the number one cause of death globally. According to the WHO, this accounts for nearly 17.9 million lives lost each year. One of the most common types of CVD is atherosclerosis. Expression of inflammatory molecules including cytokine interleukin (IL)-8, interleukin-1 beta (IL-1β), chemokine (C-C motif) ligand-2 (CCL2), chemokine (C-X-C motif) ligand-1 (CXCL1), and intercellular adhesion molecule-1 (ICAM-1) plays a vital role in triggering endothelial damage and hence significantly contributes to the initiation and progression of atherosclerosis. Carbon nanodots (CNDs) are a new class of carbonaceous nanomaterials. This study demonstrated considerable attention due to its small size, green synthesized approach, unique photoluminescent characteristics, and its great potential in drug delivery. However, the effects of CNDs on the expression of inflammatory molecules remain largely unknown. This study investigated the impact of CNDs on cell viability and tumor necrosis factor-alfa (TNF-α)-induced expression of IL-8, IL-1β, CCL2, and ICAM-1 in human microvascular endothelial cells (HMEC-1). Results demonstrated that CNDs at concentrations up to 0.3 mg/mL did not have a negative effect on cell viability in HMEC-1. Interestingly, the treatment of CNDs caused a significant decrease in TNF-α induced expression of proinflammatory molecules in HMEC-1. In animal studies, administration of CNDs also significantly suppressed TNF-α-induced increase in circulating chemokines and adhesion molecules, specifically CCL2 and CXCL1, in C57BL/6 mice. Animal body weights throughout CNDs exposure showed no significant change compared to the control group. Overall, these results help to understand the cytotoxicity and anti-inflammatory mechanism of CNDs with great insight on the potential biomedical uses of CNDs in atherosclerosis treatment.

2200 Sex Differences in Lung Inflammatory Responses to Nickel Nanoparticles In Vivo and In Vitro

Nickel nanoparticles (NiNPs) are widely used in various technological applications. A strong association between nickel inhalation exposure and asthma, pulmonary fibrosis, and lung cancer has been established in humans. In general, epidemiology studies show that women are more susceptible than men to chronic lung inflammation, while men are more susceptible to acute lung inflammation. The goal of this study is to explore mechanisms of susceptibility between male and female mice to lung inflammation after NiNP exposure in vivo and the effect of estradiol treatment on NiNP-induced cell signaling and pro-inflammatory cytokine production in human lung epithelial cells in vitro. C57BL/6J mouse female mice were treated by oropharyngeal aspiration of NiNPs (14 μg/cm2), LPS (5 μg/kg or 0.83μg/kg), NiNPs (14 mg/kg or 0.67 mg/kg), or both for the acute and subchronic study. Human bronchial epithelial cells (BEAS-2B) were stimulated with NiNPs (3 μg/cm2), LPS (25 ng/mL), or both, with or without 17β-estradiol treatment or TP-CA1 (5-(p-Fluorophenyl)-2-ureido) thiophene-3-carboxamide), an IKK2 inhibitor, treatment. The in vitro study showed that male mice were more susceptible to lung inflammation with both the acute and subchronic exposure to LPS and NiNPs. Our data suggest that the mechanism of male susceptibility to acute NiNP-induced lung inflammation involves increased neutrophil infiltration regulated by IL-6/STAT3 whereas male susceptibility to subchronic lung inflammation involves monocyte infiltration regulated by CCL2. The in vivo study showed that NiNPs increased LPS-induced production of IL-6 secreted protein levels which was suppressed with both 17β-estradiol and TP-CA1 treatment. TP-CA1 treatment suppressed STAT3 activation specifically while NF-kB and ERK activation levels stayed the same. Thus, our data suggest that the synergistic induction of IL-6 LPS and NiNPs treatment involves the STAT3 signaling pathway in vitro. However, no changes in STAT3 phosphorylation were seen in 17β-estradiol treated BEAS-2B cells, suggesting other mechanisms(s) could play a role in the
suppression of IL-6 in 17β-estradiol treated cells. Future in vitro studies will explore the mechanism of suppressed IL-6 protein levels upon exposure to 17β-estradiol in cells. Supported by NEIHS grant R01-ES020897, NEIHS Training Grant T22ES007046, and NSF Grant CBET-1530505.

2201 Comprehensive Meta-Analysis of Toxicogenomics Datasets Reveals Engineered Nanomaterials Mechanism of Action
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Engineered nanomaterials (ENMs)-enabled products have increasingly emerged in the last decades. However, the investigation of their toxicological effects and mechanism of action (MOA) has lagged behind their development and diffusion. Toxicogenomics approaches allow the characterization of the complex mechanisms of molecular adaptation occurring in the biological systems upon exposure. The meta-analysis of multiple toxicogenomics studies of different classes of ENMs in vitro and in vivo can help to identify common and specific molecular mechanisms of action. To date, however, the role of genetic and epigenetic characteristics of the biological systems in defining specific responses is still largely unknown. We meta-analyzed a comprehensive collection of 71 transcriptomics datasets derived from multiple human and mouse tissues and cell types exposed to ENMs, as currently curated by Saarimäki et al. We defined a data space of 491 exposure conditions (treatment, exposure time and dose, and biological system) and 3,699 genes. By this approach we ranked the genes by their patterns of molecular alterations across different nanomaterial exposures. We identified genes associated with ENM MOA and related these findings with structural characteristics of the gene promoter regions, hence suggesting possible epigenetic factors influencing specific responses to exposure. Our approach facilitates the use of toxicogenomics in resolving the ENM MOA, and further opens new avenues to precision toxicology.

2202 Occupational Health and Safety Implications of Nano-Enabled Building Materials during Sanding and Incineration
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The widespread use of engineered nanomaterials (ENMs) in building materials such as paints, coatings, facades, and insulation materials raises uncertainties about occupational exposures and potential health implications during their lifecycle or end-of-life. The present study investigates potential ENM release from two industrially relevant families of building materials, i.e., in-house synthesized nanocoatings (acrylic polymer matrix with nano Fe3O4 or DPP-Red organic nanofiller) and two commercially available nano-enabled insulations, during relevant lifecycle scenarios: 1) sanding, 2) incineration, and 3) incineration w/ and w/o prior UV-aging of. The released inhalable PM fraction was sampled and used to assess the potential acute biological adverse effects and cytotoxicity of the released particulate matter (PMi). Calu-3 human lung epithelial cells were exposed to occupationally relevant doses of 20 and 75 µg/mL for a 24-h exposure. Supernatants from exposed cells were also analyzed for inflammatory cytokines/chemokines, while the cell lysates were used for RNA-seq-based transcriptomic profiling, to investigate possible toxicological mechanisms and gene pathways for disease. Detailed physicochemical and morphological characterization of the released PM confirmed the presence of nanoscale metals/metal oxides originally in the coatings and insulations. No significant change in the cellular viability or oxidative stress was observed for any of the materials or lifecycle scenarios irrespective of dose, however a statistically significant increase in metabolic activity was observed at the higher dose for the incinerated Fe3O4-enabled nanocoating, accompanied by significant upregulation of inflammatory biomarkers, such as TGFα, TGFβ, PDGF-AA, and VEGF-A, and suppression of IP-10, suggesting a possible role in the development of neangiogenesis and collagen deposition. Overall, this work highlights the importance of considering real-world PM exposures from nano-enabled building materials for occupational health risk assessments and warrants further investigation into the possible acute and chronic health effects of such exposures.

2203 Dissolution of Citrate Stabilized, Polyethylene Glycol Coated, Carboxyl and Amine Functionalized Gold Nanoparticles in Simulated Biological Fluids and Environmental Media
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Dissolution is an important property utilised to eludicate both short- and long-term effects of nanoparticles for their potential to cause harm to humans and the environment. Nanoparticles may therefore be classified based on their (bio)durability between those that are amenable and those that are resistant to dissolution, biodegradation and/or disintegration. The dissolution kinetics of uncoated citrate stabilized, polyethylene glycol coated (PEGylated) gold nanoparticles functionalized with carboxyl and amine functional groups in simulated biological and environmental fluids at physiological and room temperature, respectively were studied using the static dialysis protocol to predict their (bio)durability. Citrate stabilized gold nanoparticles showed high degrees of resistance to dissolution in the simulated media unlike those, which were coated with polyethylene glycol and functionalised with carboxyl and amine functional groups. Generally, the extent of AuNP dissolution in acidic media (phagolysosomal fluid and gastric fluid) was greater than in neutral or alkaline media such as Gamble’s fluid, blood plasma and intestinal fluids, freshwater and seawater. However, in all these experimental conditions, the particles did not completely dissolve. In the case of amine functionalised AuNPs, the nanoparticles released a maximum of only 15% of their original concentration whereas carboxyl functionalized, and citrate stabilized gold nanoparticles released 9% and 8.5% of gold ions, respectively. Rate and degree of dissolution depended on the surface functionalization, pH, ionic strength of the simulated fluid and particle aggregation. Therefore, the results indicate that gold nanoparticles with low dissolution rates are expected to be (bio)durable in biological and environmental surroundings thus they might impose long-term effects on humans and the environment. In contrast, those with high dissolution rate are not (bio)durable and hence may cause short-term effects.

2204 Simulated Gastric Digestion and In Vivo Intestinal Uptake of Orally Administered CuO Nanoparticles and TiO2 E171 in Male and Female Rat Pups
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Oral exposure to nanoparticles (NPs) during early life is an understudied area. The goals of this study were to evaluate the effect of gastric fluids on NPs in vitro, and to evaluate uptake in vivo. Simulated in vitro gastric digestion of 50 supracritical CuO and TiO2 E171 was performed for developmental stages in pre-weaned rat pups. Digested NPs were characterized using dynamic light scattering, scanning electron microscopy, and inductively coupled plasma atomic emission spectroscopy. The intestinal NP uptake was studied in vivo in male and female Sprague-Dawley rat pups following oral administration of nanoparticles at a single daily dose of 10 mg/kg CuO NPs, TiO2 E171, or vehicle control (water) between postnatal days (PND) 7-10. Rat pups were sacrificed on either PND 10 or PND 21 and selected tissues were collected, weighed, and processed for histopathology. Intestinal tissue sections were evaluated by bright field microscopy, Enhanced Darkfield Microscopy, and Hyperspectral Imaging to evaluate NP uptake and the number of immune cells. Simulated gastric digestion led to dissolution of CuO NPs at the later developmental stages (bland phase (−PND 7): 3.5%; transitional phase (−PND 14): 17%; acidic phase (−PND 21): 78%). No detectable dissolution was observed for TiO2 E171. In vivo intestinal uptake of CuO NPs and TiO2 E171 was observed by microcopy analysis of intestinal cross sections. The number of granulocytes in the small and large intestine for both male and females were between 2- to 4-fold higher following CuO NPs and TiO2 E171 administration. For both male and female pups, the number of intraepithelial lymphocytes (IEL) was increased in the small intestine (2- to 3-fold higher), while the IEL in the large intestine were similar for female and 2-fold higher for male. Intestinal uptake of CuO NPs and TiO2 E171 in rat pups was demonstrated. Orally administered NPs led to increased number of immune cells in the small and large intestine, suggesting that oral exposure to NPs during early life may lead to irritation or a low grade of inflammation. The long-term impact of increased immune cells in the intestinal tract during early life is unknown.
Characterization of Nano-Sized Silica in Agricultural Exposures and Role in CKDu Pathogenesis
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Sugarcane burning by farmers in developing countries is hypothesized to contribute to an emerging kidney pathology called chronic kidney disease of an unknown etiology (CKDu). Furthermore, elemental analysis of sugarcane stalks and leaves shows a high percentage of amorphous silica (SiO₂). We hypothesized that burning of sugarcane generates nanosized particles that are inhaled and deposited in the kidney thereby contributing to CKDu in sugarcane workers. To determine if nanosized particles are present in sugarcane ash, we utilized single particle (SP) inductively coupled plasma-mass spectrometry (ICP-MS) and dynamic light scattering (DLS). Using SP-ICP-MS, we identified silica nanoparticles (SiNPs) within digested sugarcane ash (SA) ranging in size from 190-212 nm. DLS analysis of digested ash confirmed the presence of nanoparticles with a hydrodynamic diameter of ~112 nm. To determine the effects of SiNPs on the kidney, we used a human proximal convoluted tubule (PCT) cell line (HK-2) to recapitulate the nephron’s exposure to both sugarcane ash and different sized SiNPs. HK-2 cells were exposed to 0.25, 2.5, and 25 µg/mL 200 nm SiNP, SA, desilicated sugarcane ash (DS Ash) or sugarcane ash derived SiNPs (SAD SiNPs) for 6 hrs to examine cytotoxicity by MTS assay. Treatments were not cytotoxic to HK-2 cells after 6 hrs at the highest dose. However, we observed significant cellular uptake of SiNPs following exposure which was associated with increased lysosomal membrane permeabilization. Overall, these preliminary observations demonstrate the presence SiNPs in SA, which are readily taken up by PCT cells leading to lysosomal damage suggesting that mechanisms such as inflammatory activation may contribute to CKDu.

Cytotoxicity of Display-Inspired Quantum Dot Is Higher Than the Additive Effect of Its Components
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Quantum Dots (QDs) have become widely available in televisions and displays because of their ability to fluoresce at tunable narrow wavelength distributions. However, there has also been considerable concerns over the environmental and human health arising from the anticipated release of toxic heavy metals and other entities from disposed display panels. This study adopted a QD model based on patented commercial product that has been developed for optimal fluorescence stability with graded CdS/ZnS shells over a CdSe core. In addition, the functional QD contained a polymer coating of polyethyleneimine (PEI) complexed with 1,2 epoxy-3-phenoxypropane (E3P) for its dispersion in plastics. Complete QD (CdSe/ZnS-PEI&E3P) and combinations individual components (CdSe, CdSe/ZnS, ZnS, CdSe-PEI&E3P, ZnS-PEI&E3P, PEI&E3P) were exposed to human intestinal epithelial cells (HIEC-6) for in vitro toxicity assessment. EC20 of cell viability was taken as indicator for cytotoxic effect of individual components in defining cytotoxicity of complete QD were observed. Further, investigations suggested that complete QD containing a cationic surface polymer resulted in higher cellular uptake of Cd. The complete QDs could also initiate higher sublethal effects including oxidative stress, pro-inflammatory response, intracellular Ca²⁺ flux changes and mitochondrial dysfunctions that can be attributed to the metal and ligand components. Therefore, the synergistic effects that cause the higher toxicity of complete QDs was an interplay between the toxicity pathways of individual components, facilitated by the increased bioavailability due to surface chemistry.

A Decade of Toxicological Trends: What the Papers Say
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Here we look at popular trends and concepts in toxicology over the decade 2009-2019. The top 10 concepts included methodological approaches such as zebrafish and genomics as well as much broader concepts such as personalisation of medicine and adverse outcome pathways (AOPs). The total number and rank order for each of the top 10 was tracked year by year via PubMed. The data revealed a slow upward trend in the number of papers across all the concepts from 260 in 2009 to more than 1700 papers in 2019. Zebrafish, genomics and personalised medicine remained in the top four slots since 2009 with zebrafish dominating the rankings over the entire decade. Genomics was a strong second until 2013 when it was displaced first by the microbiome in 2014 and secondly by personalised medicine in 2015. Other notable trends were the ascendency of the microbiome and AOPs and the descendency of hormesis and the 3Rs (replacement, reduction and refinement of animals in testing). The observation that the top 4 slots have been static over the past 4 years as well as that new ideas are introduced and increase in popularity until they find their place in scientific culture. This may suggest that relatively new concepts such as artificial intelligence (AI) and microbiological systems (MPS) have yet to find their steady state in the rankings. Similarly, as a relatively new player in the toxicology field, the full impact of the human microbiome on drug efficacy and safety remains to be seen. This study also showed that in top ranking concepts between tools/techniques such as genomics, zebrafish and read-across with broader concepts such as the microbiome and personalised medicine. Regarding future trends, newer tools/techniques such as CRISPR-Cas9, image analysis and next generation sequencing (NGS) may feature heavily in the general published literature but not yet in toxicology publications. This suggests that it takes time for these trends to be picked up and used in our field.

Multi-tissue Transcriptomic Analysis of Diversity Outbred Mice Fed a High-Fat Diet
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Individual susceptibility is a critical modifier of chemical toxicity and risk. Given that obesity affects over 30% of the US population, it is important to develop systems that interrogate the interactions between this underlying condition and chemical toxicity. Ketogenic diets and metabolic diseases are complex and there is strong evidence that interindividual genetic variability influences health outcomes. Recent findings from the clinic suggest the presence of subgroups within metabolic diseases such as diabetes; molecular characterization of these metabolic subgroups will enable personalized treatment and prevention. We hypothesized that the Diversity Outbred (DO) mouse, which have high genetic variability, could be utilized as a model for understanding human variability in metabolic diseases, providing insight both into chemical susceptibility associated with metabolic disease and into the molecular characterization of metabolic subgroups. Male DO mice were fed a control diet or high-fat diet (HFD; 60% kcal from fat) for 13 weeks. Animals fed HFD on average gained more weight (mean ± SD: 20 ±7 vs. 10 ± 4 g body weight gain), had hyperglycemia (mean ± SD: 237 ± 87 vs. 180 ± 37 mg/dL) and impaired glucose tolerance compared to control animals at study end. Classification and regression tree machine learning analyses identified 12 subgroups in the population based on body weight gain. To investigate molecular signatures and potential systems-level transcriptomic biomarkers of these subgroups, RNA-sequencing was conducted on liver, adipose, and muscle, key tissues in glucose homeostasis. Notably, gene expression profiles distinguished the metabolic subgroups. Metabolic subgroups with larger differences in body weight gain were also differentially expressed genes in the adipose and liver. Overall, these findings suggest that there are metabolic subgroups in the DO mouse population with variations in molecular signatures. Future research activities will investigate specific transcriptomic biomarkers for these metabolic subgroups and identify potential mechanisms and biomarkers of susceptibility to chemical exposures in the context of high-fat diet consumption and metabolic disease.

Thirdhand Smoke: Effects on Oxidation and the Plasma Proteome
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Thirdhand smoke (THS) is residual cigarette smoke that lingers on indoor surfaces after a cigarette has been extinguished. Because it contains toxicants including carcinogens, it is a health concern. Our goal was to determine if dermal exposure to THS increases oxidation of urinary biomarkers and alters the plasma proteome. We recruited 10 healthy non-smoking human subjects into a cross-over study. Participants wore either clean clothing or THS impregnated clothing for 3 hours while exercising on a treadmill in a laboratory environment. Urine samples were collected at 0, 1.5, 3, first morning collection, and 24 hours. Blood samples were taken at 0, 3, and 24 hours. The subject's urinary biomarker analysis measured by ELISA demonstrated significantly elevated oxidative damage to DNA (8-OHdG), lipids (8-isoprostane) and proteins (protein carbonyls). Plasma proteomics was performed in an Orbitrap Fusion Lumos Tribid Mass Spectrometer (ThermoFisher) and pathway analysis was
performed using Ingenuity Pathway Analysis (IPA) (Qiagen). After THS exposure, 3 subjects showed significant fold changes (±1.5 log2-fold change) in 31 plasma proteins at 3 hrs and 34 proteins at 24 hours when compared to 0 hours samples. At 3 hours of THS exposure activated canonical pathways (z-score ≥2.0) included migration of cells, blood homeostasis, and movement of immune cells. At 24 hours post-THS exposure, activated pathways included cell movement, movement of phagocytes, increased angiogenesis and vasculogenesis. Reactome pathway analysis of THS 3 hours exposure identified 68 significant (p-value ≤0.05) pathways, which included ECM proteoglycans, neutrophil and platelet degranulation, integrin cell-surface interactions, and VEGF ligand-receptors binding. The THS 24 hours post-exposure sample had 36 significant pathways which included activation of the innate immune system and complement cascade and glycolysis alterations. Our study demonstrates that an acute exposure to THS increases oxidation of urinary DNA, lipid, and proteins and alters the plasma proteome. Cigarette smoke also increases oxidative stress and alters some of the same pathways (e.g., activation of the immune system, blood homeostasis, and altered cell migration). This is the first clinical trial to demonstrate adverse responses in humans exposed dermally to THS smoke.

2211 Predicting the Impact of Cholehepatic Shunt of Bile Acids on Cholestatic Liver Injury Using Quantitative Systems Toxicology Modeling
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Cholestatic liver injury can be caused by impaired function of efflux transporters located on the canalicular membrane of hepatocytes. While xenobiotic-induced inhibition of the bile salt export pump (BSEP) has been extensively shown to result in toxic accumulation of bile acids in hepatocytes, increased biliary flow, there is increasing evidence supporting an important, protective role for multidrug resistance protein (MDR) 3 function in cholestatic hepatotoxicity. Inhibition of the phospholipid floppase MDR3 leads to reduced bile acid-phospholipid mixed micelle formation and an elevated biliary bile acid-phospholipid ratio that is toxic to the epithelial cells lining the biliary tree. The cholehepatic shunt is an understudied pathway for bile acid disposition and may play a significant role in adaptation to cholestatic liver injury, particularly when there is an excess of free biliary bile acids. In the current study, the existing bile acid and phospholipid sub-models within the quantitative systems toxicology modeling and simulation platform DILIsym X were extended to explicitly represent distinct regions of the biliary tree, including the hepatic duct, common hepatic duct and the common bile duct. Simulations were performed to assess the impact of the cholehepatic shunt on bile acid disposition, and on the bile acid-phospholipid ratio, specifically when MDR3 function is impaired. Simulations showed that activation of the cholehepatic shunt substantially decreases biliary concentrations of bile acids, but increases hepatic bile acid concentrations. Shunting from the hepatic duct appeared to offer the most protection to the biliary tree, while shunting in general lead to the lowest biliary acid-phospholipid ratio to the common bile duct. When MDR3 was impaired, the biliary acid-phospholipid ratio was elevated, as expected, and could be decreased by the activation of the cholehepatic shunt. The current modeling and simulation study suggests that the cholehepatic shunt may protect the biliary epithelium by reducing biliary bile acid concentrations, but may have an undesirable impact on hepatocytes by increasing the hepatic bile acid burden. The impact of bile acid shunting could have important implications in drug development, especially in the case of MDR3 inhibitors, and when the potential for drug-induced liver injury is evaluated.

2212 Spatial Temporal Online In Vitro Toxicity Testing Employing a Novel Magnetic Resonance Technique Using Thymic Carcinoma 3D Models
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The metabolic pathogenesis of thymic carcinomas (TCs) is poorly understood and adjuvant therapy has limited success in metastatic disease and tumor recurrence. In addition, anticancer therapy is only partially effective, mainly due to inherent or drug-induced resistance of tumor cells to standard chemotherapy and radiotherapy. Therefore, novel therapeutic strategies are urgently needed. Most studies on TCs use two-dimensional (2D) cell culture models, which are not considered as physiologically relevant, and consequently translation to the in-vivo situation remains challenging. Tissue-specific architecture, based on in interactions with the microenvironment is an essential component of tumors and may be better recapitulated in three-dimensional (3D) cell culture models. 3D models are proposed to be more resistant against anticancer drugs, which are more similar to the actual tumor microenvironment. The goal is to establish 3D thymoma models which will then be used to understand the pharmacokinetics and pharmacodynamics of anticancer drug therapy via metabolic profiling of living cells. We established 3D models of two thymic carcinoma cell lines, TYY2 and 1889c, in normal and serum free media using simple rotation for the former cell line which was derived from a thymic carcinoid and the latter from a non-small cell thymus carcinoma. 3D models using traditional toxicity assays, as well as in vivo imaging. A commercially available probe head was used to perform metabolic profiling of the 2D culture. In addition, a technique was established using Nuclear Magnetic Resonance (NMR) that is suitable for measuring ultra-small volume samples, like 3D tumor models in order to perform metabolic profiling in these 3D models as a function of spatial position. For the first time, 3D spheroids could be made of reproducible size and quality using two thymic cancer cell lines. We could also produce spheroids using serum free media to decrease differentiation of the cells normally seen in this cancer type. Live/dead dyes showed that the cells in the untreated spheroids were viable, with evidence of a hypoxic core the larger the spheroid. Metabolic profiling of the 2D and 3D models for both cell lines were identified for example: spheroids react to unfavorable tumor microenvironment (low nutrient, limited oxygen supply and drug treatment) by metabolic reprogramming, similar to tumor cells in vivo so that we can use these models to test for efficacy of new drugs. Moreover, we were able to determine toxicological endpoints on a cellular level and we could deduce details on the drug transport efficiency and on drug target modifications from these measurements. The present findings using 3D models of thymic cancer confirm that they are more resistant to drug-induced toxicity compared to 2D models. In addition, our novel approach using NMR allows for the measurement of small tissue-like models, such as in vitro 3D cell culture models, which are normally not feasible with standard analytical techniques. The currently-available methods only provide a “snap-shot” of the measured time point and tend to be destructive, e.g. dissecting or optical cleaning of the specimen to gain 3D Information - a limitation we overcome with our current method using NMR spectroscopy.

2213 Capillary Aerosol Generator for Continuous Production of Controlled Aerosol
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The capillary aerosol generator (CAG) concept was developed nearly 20 years ago and has found various applications, including in generation of pharmaceutical aerosols. We have developed our own setup for compact generation, dilution, and delivery of aerosols generated from the CAG. The objective of these developments was to establish and characterize a system for continuous aerosol generation from liquid mixtures present in e-vapor products for therapeutic aerosols. We have developed our own setup for compact generation, dilution, and delivery of aerosols generated from the CAG. Furthermore, we were able to determine the aerodynamic size distribution of the generated aerosol using dynamic conditions. The liquid is supplied via a pump at a controlled flow rate to a capillary, which is continuously heated beyond the boiling point of the mixture, causing bursts of vapors to be generated and discharged from the capillary orifice into a stream of cold air. The generated aerosol is then diluted with ambient cold air, triggering nucleation processes and subsequent dilution of the aerosol. Therefore, our goal is to establish 3D thymoma models which are not considered as physiologically relevant, and consequently translation to the in-vivo situation remains challenging. Tissue-specific architecture, based on in interactions with the microenvironment is an essential component of tumors and may be better recapitulated in three-dimensional (3D) cell culture models. 3D models are proposed to be more resistant against anticancer drugs, which are more similar to the actual tumor microenvironment. The goal is to establish 3D thymoma models which will then be used to understand the pharmacokinetics and pharmacodynamics of anticancer drug therapy via metabolic profiling of living cells. We established 3D models of two thymic carcinoma cell lines, TYY2 and 1889c, in normal and serum free media using simple rotation for the former cell line which was derived from a thymic carcinoid and the latter from a non-small cell thymus carcinoma. 3D models using traditional toxicity assays, as well as in vivo imaging. A commercially available probe head was used to perform metabolic profiling of the 2D culture. In addition, a technique was established using Nuclear Magnetic Resonance (NMR) that is suitable for measuring ultra-small volume samples, like 3D tumor models in order to perform metabolic profiling in these 3D models as a function of spatial position. For the first time, 3D spheroids could be made of reproducible size and quality using two thymic cancer cell lines. We could also produce spheroids using serum free media to decrease differentiation of the cells normally seen in this cancer type. Live/dead dyes showed that the cells in the untreated spheroids were viable, with evidence of a hypoxic core the larger the spheroid. Metabolic profiling of the 2D and 3D models for both cell lines were identified for example: spheroids react to unfavorable tumor microenvironment (low nutrient, limited oxygen supply and drug treatment) by metabolic reprogramming, similar to tumor cells in vivo so that we can use these models to test for efficacy of new drugs. Moreover, we were able to determine toxicological endpoints on a cellular level and we could deduce details on the drug transport efficiency and on drug target modifications from these measurements. The present findings using 3D models of thymic cancer confirm that they are more resistant to drug-induced toxicity compared to 2D models. In addition, our novel approach using NMR allows for the measurement of small tissue-like models, such as in vitro 3D cell culture models, which are normally not feasible with standard analytical techniques. The currently-available methods only provide a “snap-shot” of the measured time point and tend to be destructive, e.g. dissecting or optical cleaning of the specimen to gain 3D Information - a limitation we overcome with our current method using NMR spectroscopy.
2214 Differential Gene Expression in Liver in Rats at High Daily Doses of 2,3,7,8-Tetrachlorodibenzo-p-dioxin and Linkages between AHR-Arnt and Circadian Processes in Peripheral Organs

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Large single doses of TCDD in rats (~ 100 µg/kg) causes wasting, a progressive body weight loss of nearly 30% leading to death in several weeks. A persistent PPARα agonist, perfluoro-n-decanic acid, causes wasting after doses of about 30 mg/kg. We evaluated numbers of differentially expressed genes (DEGs) in peripheral (PP) and centrilobular (CL) regions of the liver in rats dosed with 0, 3, 22, 100, 300 or 1000 ng TCDD/kg/day, 5/day/week for four weeks and examined pathway enrichment with Reactome. There were dose-dependent reductions in cell proliferation and significantly reduced body weight-gain at the highest dose. There were larger numbers of DEGs in the CL region of the dose response was left-shifted compared to the PP region. The numbers of DEGs (FC>1.5; FDR<0.05) across doses for CL (PP) were 3 (1), 45 (2), 38 (38), 813 (263) and 1936 (901). Despite different dose responses, the pathway responses at 1000 ng/kg/day in CL and PP were similar with profound down-regulation of multiple metabolism pathways - cholesterol biosynthesis, fatty acid metabolism, signaling by nuclear receptors and PPARα activates gene expression - and upregulation of ECM proteoglycans, unfolded protein response and elastic fiber and collagen formation. A subset of “PPARα activates gene expression” pathway genes were upregulated in the PP region. From these results, we (1) propose a hypothesis for Ah-Arnt in circadian control of cellular metabolism where the Ah-Arnt dimmer limits cell-proliferation and activates CYPs to metabolize dimerization-inducing ligands while the free forms of Ah-Arnt and Arnt have permissive roles in proliferation and vascularization and (2) provide a schematic for Ah-Arnt control of peripheral circadian rhythms, involving light induced Ah-Arnt ligands, melanin clearance by Ah-Arnt regulated CYPs and cycling between bound and free forms of Ah-Arnt. Both TCDD and persistent PPARα ligands likely function through similar circadian-linked pathways to cause wasting. These studies were supported by Dow Chemical Company.

2215 Thioesterase Induction by 2,3,7,8-Tetrachlorodibenzo-p-dioxin Elicits a Futile Cycle That Inhibits Hepatic β-oxidation

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Non-alcoholic fatty liver disease (NAFLD) includes a spectrum of disorders ranging from simple steatosis, characterized by excessive lipid accumulation to non-alcoholic steatohepatitis (NASH) with fibrosis. Diverse pharmaceuticals and xenobiotics induce hepatic fat accumulation including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds. Lipidomic analysis of liver extracts from TCDD-treated mice identified marked increases in unsaturated fatty acids (FA), triacylglycerols, and cholesterol esters. In the present study, β-oxidation of straight chain FAs was examined in mice treated with 0.01, 0.03, 0.1, 0.3, 1, 3, 10, or 30 µg/kg body weight TCDD every 4 days for 28 days to test the hypothesis that TCDD represses gene expression associated with β-oxidation. Untargeted metabolomic analysis revealed 30 µg/kg TCDD decreased hepatic decanoyl-, hexanoyl-, butyryl-, and acetyl-CoA levels 126.6-, 34.9-, 11.8-, and 6.3-fold, respectively, while inducing octanoyl-CoA 138.9-fold. Bulk RNA-sequencing suggested TCDD repressed the hepatic gene expression associated with lipolysis, binding proteins (i.e., Fapb1, Dbi, and Scp2), FA activation, and thiolysis while inducing thioesterases. Protein levels for the binding protein DBI, the FA activators ACSM3 and ACSL1, and the acyl-CoA dehydrogenase ACOX1, all exhibited dose-dependent repression. During β-oxidation, acyl-CoAs are subjected to oxidation, hydration, a second oxidation and finally, coenzyme A (CoASH)-dependent thioleavage to produce acetyl-CoA and an acyl-CoA that is two carbons shorter. Decreased acyl-CoA levels and the accumulation of medium chain enoyl-CoA species was consistent with incomplete oxidation. Collectively, the integration of metabolomics and RNA-seq data suggested TCDD induced a futile cycle of fatty acid activation and acyl-CoA hydrolysis resulting in incomplete β-oxidation. GNC and RRF are supported by the NIEHS Multidisciplinary Training in Environmental Toxicology Program (T32ES007255). This project is funded by the Superfund Research Program (P42ES004911).
Flame retardant chemicals (FRCs) commonly added to many consumer products present a human exposure burden with a potential to produce adverse health effects. Under pressure from consumers, FRC manufacturers have adopted some purportedly safer replacements for first-generation brominated diphenyl ethers. In contrast, second and third-generation organophosphates and other alternative chemistries have limited bioactivity data available to estimate their hazard potential. In order to evaluate the toxicity of existing and potential replacement FRCs, we need efficient screening methods. We built a 61-FRC library in which we systemically assessed developmental toxicity and potential neurotoxicity effects in the embryonic zebrafish model. Data were compared to publicly available data generated in a battery of cell-based in vitro assays from ToxCast, Tox21, and other alternative models. Of the 61 FRCs, 19 of 45 that were tested in the ToxCast assays were bioactive in our zebrafish model. The zebrafish assays detected bioactivity for 10 of the 12 previously classified developmental neurotoxic FRCs. Developmental zebrafish were sufficiently sensitive at detecting FRC structure-bioactivity impacts that we built a classification model using 13 physicochemical properties and 3 zebrafish assays that achieved a balanced accuracy of 91.7%. This work illustrates the power of a multi-dimensional in vivo platform to expand our ability to predict the hazard potential of new compounds based on structural relatedness, ultimately leading to reliable toxicity predictions based on chemical structure.

Crassoeverus rubens (C. rubens) is a tropical African and Indian vegetable consumed as soup and sauce in Western Nigeria. It is also used in folklore medicine in the management of stomach-ache, liver complaints and as an antidote for poison. In this study, we evaluated the hepatoprotective activity of the aqueous extract of C. rubens (AQLE) on PAAc-induced toxicity. Twenty-four male Wistar rats were randomly distributed into four groups of six animals each. Group I received normal saline, groups II, III and IV received PAAc (20 mg/kg) intraperitoneally, AQL (500 mg/kg) orally and PAAc (20 mg/kg) + AQL (500 mg/kg) respectively for two weeks. Thereafter, we determined Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) activities in the serum. In addition, Glutathione and Nitric Oxide (NO) levels as well as activities of Catalase (CAT), Superoxide Dismutase (SOD), Glutathione-S-transferase (GST), and Xanthine Oxidase were determined in the liver. The results showed increased activities of ALT, AST and ALP in the serum of rats treated with PAAc as compared with control. We observed significant reduction in the activities of CAT and XO (p < 0.05). However, PAAc ameliorated PAAc-induced elevation of ALT, AST and ALP and reduction in XO and CAT activities (p < 0.05). Lastly, lesion induced by PAAc was ameliorated by AQLE. In conclusion, our data provided further insights on antioxidative potential of AQL and as a possible regimen for the prevention/treatment of oxidative stress-mediated liver injury induced by lead and related heavy metals.
2223 Investigating Proteomic and Epigenetic Changes in Alcohol-Associated Hepatitis

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Alcohol consumption remains the leading cause of liver disease worldwide and is one of the greatest sources of preventable mortality. Alcohol-associated liver disease (ALD) comprises a wide range of pathologies, including steatosis, steatohepatitis, alcoholic hepatitis (AH), cirrhosis, and hepatocellular carcinoma. The rapid onset of AH in some patients requires swift action in the form of liver transplantation. Importantly, the metabolic mechanisms contributing to AH remain underdetermined and further characterizing biochemical factors that lead to AH may provide insight toward therapeutic interventions which may restore hepatic function. Over the last decade, numerous reports utilizing rodent models have shown that alcohol metabolism alters hepatic protein acetylation, including mitochondrial and nuclear proteomic targets. In order to investigate proteomic and epigenetic markers due to AH, human liver explants were obtained to determine alterations in proteomic and epigenetic marks in AH patients. Immunohistochemical analysis revealed that lysine acetylation was decreased in AH compared to controls. In other regions of AH livers a known marker for epigenetic modification, histone H3K9 acetylation was increased in the AH liver explants and three other lysine residues were trending toward increases in AH patients. Proteomic and acetylation analysis was performed utilizing nHPLC-MS/MS which revealed significant changes to proteins in metabolic pathways. The novel software xMWAS was used to integrate and visualize the epigenetic, proteomic, and acetylation data sets. The greatest decreases in protein abundance were found in metabolic pathways, fatty acid degradation, biosynthesis of amino acids, and carbon metabolism. In contrast, the largest increases in protein abundance were observed in pathways such as focal adhesion and regulation of actin cytoskeleton, bacterial invasion of epithelial cells, and alcoholism. Protein acetylation, adjusted for protein abundance, significantly decreased in pathways relating to metabolic pathways, fatty acid degradation, biosynthesis of amino acids, and carbon metabolism. Indeed, regarding end stage liver disease, metabolic changes associated with liver homeostasis are dramatically, and negatively, impacted in AH. The proteomic and epigenetic investigation of AH tissue samples, in comparison with non-diseased liver tissue, demonstrates an innovative translational approach toward understanding pathological mechanisms of hepatocyte metabolism, proteomic changes, and epigenetic marks during the progression and development of AH.

2225 Alarmin IL33 Is Vasoconstrictive and Acutely Disrupts Endothelial-Dependent Dilatation

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Critical discoveries in the past demonstrate inhalation of environmental or industrial toxins stimulate cardiovascular dysfunction. Our group is particularly interested in the microvascular health issues associated with exposure to nano-titanium dioxide (TiO₂). TiO₂ is biopersistent and widely used in advanced materials from filters to surface coatings, which greatly increases the chance of exposure. Previously, we identified a pulmonary burden (100 μg) of TiO₂ mediated vascular impairment, which was blunted by neutralization of the alarmin IL33. However, the mechanisms of IL33-mediated vascular impairment remain unexplored. Thus, we aimed to determine if IL33 directly mediates TiO₂-induced vascular impairment. 2nd order mesenteric arteries and aortic rings were excised from male Sprague Dawley rats and mounted on glass pipettes and wire myograph respectively. Arterioles and aortic rings were excised from male Sprague Dawley rats and mounted on glass pipettes and wire myograph respectively. IL33 exhibited a dose-dependent vasoconstrictive response to IL33 (p<0.05), with maximum constriction of 11±2 μm. Additionally, 2-hour incubation with 1 ng/ml of IL33 impaired acetylcholine-induced vasorelaxation in aortic rings (max relaxation 98±2% control vs. 80±5% IL33) and arteries (max relaxation 51±8% control vs. 19±5% IL33). At the cellular level ERK signaling is activated in endothelial cells increasing 3-fold with-in 5 minutes (p<0.01) and remaining elevated at the 30-minute time point (5-fold increase from baseline, p<0.01). Mechanistically, endothelial ERK signaling has been linked to eNOS inhibition and release of endothelin-1, which are currently being explored. Our results demonstrate that IL33 inhibits aminopeptidase N, a major net peptidease responsible for IL33 degradation. Alarmins exhibit a complex regulation of vascular homeostasis and future studies should address the role of IL33 in the regulation of this important pathway in vascular health.

2226 Nanomaterial Inhalation during Gestational Windows Alters Maternal Angiotensin II Microvascular Sensitivity and Fetal Outcomes

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Engineered nanomaterials provide great benefits to quality of life and human health, yet the cardiovascular and reproductive consequences associated with exposure are not fully known. We aimed to determine the sensitive window of gestation when adverse microvascular effects begin and when the greatest detriment to fetal health occurs following maternal inhalation exposure to nano-titanium dioxide (nano-TiO₂). Our hypothesis was that specific time-points of gestational exposure would lead to varying degrees of microvascular dysfunction. Angiotensin II (ang II) was utilized to assess uter-
2227 High-Fructose Diet Increases Cardiac Work Rate and Arterial Stiffness after a Single Wood Smoke Exposure in Wistar-Kyoto Rats

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Wildfires have become a concerning environmental and health problem, particularly in certain parts of the U.S. Although data show that exposure to biomass smoke leads to adverse health impacts, it is still unclear how certain diets affect these responses. This study examined the effect of a high-fructose (HF) diet on cardiovascular function and subsequent responsiveness to wood smoke inhalation in rats. We hypothesized that HF diet would alter cardiovascular mechanical performance (e.g., work rate, force generation) and cause dysrhythmia. Eight-week-old Wistar-Kyoto rats were placed on either a normal (ND) or HF diet for seven weeks and then exposed to either filtered air (FA) or 5 mg/m^3 of flaming eucalyptus wood smoke (WS) for one hour. One day after exposure, rats were anesthetized, implanted with an intraventricular Millar probe and then challenged with dobutamine to determine cardiac and arterial function under exercise-like conditions. HF significantly increased heart rate, stroke volume and cardiac output. Subsequent exposure to WS significantly increased aortic pressure in both diets and left ventricular pressure (LVP) in HF rats during the dobutamine challenge. HF rats had significantly higher stroke work when compared to ND while WS worsened arteriolar stiffness in both diets. Tau or time of active relaxation was the same in all groups. However, the underlying contributory factors of neutrophils to cardiac remodeling post MI remain unknown. The hypothesis is that augmented aged neutrophil infiltration into the heart contributes to adverse cardiac remodeling in mice. We examined neutrophil infiltration into the heart and cardiac function and left ventricle (LV) remodeling in C57BL/6J MI model created by permanent coronary ligation at different zeitgeber time (ZT). We found that cell surface markers (CD62L, CXCR2, CXCR4) of neutrophils in peripheral blood lost their diurnal oscillation 24 h post MI. Meanwhile, some clock gene expression also displayed disrupted diurnal patterns. Flowcytometry showed augmented neutrophil (CD11b^+Ly6G^-Ly6C^-) infiltration into the heart along with increased circulating aged neutrophils in all MI groups with more infiltration into the heart at ZT 5 (p<0.05), but at late stage showed no difference for aged neutrophil infiltration at different ZT points. Furthermore, infiltrating neutrophils had significantly higher CXCR2 gene expression (p<0.05). Mice that underwent ligation at ZT 5 had high mortality rate and large infarct size. Echocardiography showed those mice had significantly larger LV end diastolic and systolic volume (LVEDV; LVESV) and lower ejection fraction (LVEF) (p<0.05). Histological and Immunofluorescence staining revealed that those mice displayed more fibrosis and cardiomyocyte hypertrophy and less angiogenesis comparing to sham and ZT 13 or ZT 21 groups (p<0.05). However, treatment with anti-CXCL2 antibody in ZT 5 MI group significantly reduced LV dilatation, fibrosis, hypertrophy and improved cardiac function. These results indicate that greater aged neutrophil infiltration into the heart contributes to cardiac hypertrophy, fibrosis, and dysfunction which suggests that blocking neutrophil aging may be a therapeutic alternative following acute myocardial infarction.

2228 Sex-Based Differences in Doxorubicin-Induced Cardiotoxicity: The Role of Apelin-APJ Pathway


Differential susceptibility for the development of cardiotoxicity induced by the anti-cancer drug, doxorubicin (DOX), has been reported between males and females. However, the precise mechanism by which DOX provokes differential cardiotoxicity between the sexes is not fully known. To address this, next generation RNA sequencing (RNA-seq) was performed in hearts of adult male and female C57BL/6J mice a week after receiving a clinically-relevant 24 mg/kg total cumulative DOX dose (3 mg/kg body weight DOX or an equivalent volume of saline (SAL) given intravenous once a week for eight consecutive weeks). Additionally, hearts were examined microscopically for lesions and plasma samples were analyzed for concentrations of the myocardial injury marker (cardiac troponin T; cTnT) and late mice. Results indicated a greater cardiotoxicity in male mice as evidenced by the presence of cytoplasmic vacuolization in cardiomyocytes and 3.6-fold higher cTnT concentration in plasma compared to female mice. The RNA-seq data showed 216 genes in males and 18 genes in females were differentially expressed (False Discovery Rate <5% and ±1.5-fold change) by DOX treatment compared to concurrent SAL-treated controls. In male hearts, DOX treatment significantly increased the transcript levels of genes involved in fibrosis (Tgfβ1, Tgfβ3, Spink1, Serpine1, and Timp1; 1.8- to 2.3-fold), whereas only a modest upregulation was observed in Tgfβ1 and Timp1 (1.6-fold) in (Apelin and its receptor, APJ) were significantly upregulated (>1.2-fold) by DOX. Given that the Apelin-APJ axis has an anti-fibrotic role in the heart, DOX-related inactivation of the apelin signaling pathway in male hearts could be partly responsible for greater susceptibility of male hearts to DOX toxicity. Conversely, upregulation of genes encoding APLN and APJ proteins might have conferred cardioprotection against DOX toxicity in female mice.

2229 Aged Neutrophils Contribute to Late Remodeling Post-Myocardial Infarction

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Neutrophils, as the major mediator of anti-microbial defense and vascular inflammation, follow a diurnal regime after being released from bone marrow with fresh neutrophils peaking at nighttime and aged neutrophils peaking at daytime in mice. Experimental data also showed that myocardial infarction (MI) outcome is associated with the time-of-day of infarction. However, the underlying contributory factors of neutrophils to cardiac remodeling post MI remain unknown. The hypothesis is that aged neutrophil infiltration into the heart contributes to adverse cardiac remodeling in mice. We examined neutrophil infiltration into the heart and cardiac function and left ventricle (LV) remodeling in C57BL/6J MI model created by permanent coronary ligation at different zeitgeber time (ZT). We found that cell surface markers (CD62L, CXCR2, CXCR4) of neutrophils in peripheral blood lost their diurnal oscillation 24 h post MI. Meanwhile, some clock gene expression also displayed disrupted diurnal patterns. Flowcytometry showed augmented neutrophil (CD11b^+Ly6G^-Ly6C^-) infiltration into the heart along with increased circulating aged neutrophils in all MI groups with more infiltration into the heart at ZT 5 (p<0.05), but at late stage showed no difference for aged neutrophil infiltration at different ZT points. Furthermore, infiltrating neutrophils had significantly higher CXCR2 gene expression (p<0.05). Mice that underwent ligation at ZT 5 had high mortality rate and large infarct size. Echocardiography showed those mice had significantly larger LV end diastolic and systolic volume (LVEDV; LVESV) and lower ejection fraction (LVEF) (p<0.05). Histological and Immunofluorescence staining revealed that those mice displayed more fibrosis and cardiomyocyte hypertrophy and less angiogenesis comparing to sham and ZT 13 or ZT 21 groups (p<0.05). However, treatment with anti-CXCL2 antibody in ZT 5 MI group significantly reduced LV dilatation, fibrosis, hypertrophy and improved cardiac function. These results indicate that greater aged neutrophil infiltration into the heart contributes to cardiac hypertrophy, fibrosis, and dysfunction which suggests that blocking neutrophil aging may be a therapeutic alternative following acute myocardial infarction.

2230 Effects on the Cardiovascular System of Ex Ovo Chicken Embryos Exposed to Tris (2-chloroethyl) Phosphate (TCEP)

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Tris (2-chloroethyl) phosphate (TCEP) is one of pervasive organophosphate flame retardants (OPFRs) that have been used in textiles, industrial materials, and furniture. TCEP has been detected from tissues and eggs of wild birds. Though it has been reported that OPFRs elicit cardiotoxic effects such as decrease in the heart rate in zebrafish embryos, there are no reports regarding effects on the cardiovascular function in birds exposed to OPFRs. In this study, we assessed the cardiovascular toxicity of TCEP exposure on chicken embryos in the shell-less incubation system. Fertilized chicken (Gallus galus domesticus) eggs were treated with 50, 250 and 500 nmol/g egg of TCEP (TCEP-L, M and H, respectively) or DMSO (control) at the beginning of incubation. The heart rate (beats per minute; bpm) was significantly decreased on day 4 in TCEP-M (176.9±5.2; Mean±SEM) and TCEP-H (182.8±5.0) compared with control (205.1±3.8). The bpm of the TCEP-H group (217.6±6.6) on day
5 was also significantly decreased compared with control (241.8±2.7). The heart weight to body weight ratio was significantly increased on day 9 in the TCEP-H group compared with control. This result suggests that TCEP exposure induces cardiac hypertrophy in chicken embryos. The length and number of extra-embryonic blood vessels were significantly decreased on day 4 in TCEP-M and TCEP-H embryos. Thus, exposure of early avian embryos to TCEP may induce circulatory dysfunction by failure. In the cardiac transcriptome analysis, analyses of KEGG pathways related to cardiac muscle contraction showed the dysregulation of genes related to Ca²⁺ transport and muscle filament sliding. As a result of quantitative RT-PCR, the gene expression levels of myosin receptor 2 (RYR2), myosin regulatory light chain 2 (MYL2), and myosin light chain 3 (MYL3) were decreased by TCEP exposure. Transcription factor enrichment analysis activated NFKB1, SP1, SP3, SMA4D, GLI1, and CTNNB1 by TCEP exposure as critical molecular initiating events. Peroxisome proliferator-activated receptor gamma (PPARγ) was also enriched as a relevant transcription factor. These results indicate that TCEP exposure to chicken embryos may activate several transcription factors, including PPARγ, and alter the expression of genes related to the myocardial contraction, consequently leading to decreased heart rate and cardiac hypertrophy.

**Cardiotoxicity of Sunitinib in the B6C3F1 Mouse Model**


Sunitinib is a tyrosine kinase inhibitor (TKI) used to treat cancers such as metastatic renal cell carcinoma, gastrointestinal stromal tumor, and pancreatic neuroendocrine tumors. Even though sunitinib extends the survival of cancer patients, various clinical studies indicated that it can cause adverse cardiotoxic events such as left ventricular dysfunction, hypertension, QT interval prolongation and heart failure. The precise mechanisms involved in these cardiotoxicities are not fully understood. In addition, there are no clinical biomarkers currently available to predict the early onset of sunitinib-induced cardiotoxicity during cancer therapy. To address the knowledge gap, both male and female B6C3F1 mice were treated with 80 mg/kg/day of sunitinib or vehicle through oral gavage daily for 21 days. The cardiac function was measured using Vevo3100 ultrasound imaging system 24 hours after dosing at days 0, 3, 6, 9, 12, and 21, and blood samples were collected for measurements of the myocardial injury marker (cardiac troponin-I (cTnI)) and microRNAs (miRNAs). The results showed that the expression of cTnI was increased in both male and female mice indicating that sunitinib did not cause any overt cardiac injury with 21 days sunitinib treatment. However, left ventricular ejection fraction (LVEF) and fractional shortening (FS) were decreased significantly (p<0.05) at day 9 in males and at day 6 in females after sunitinib treatment with the highest decline in LVEF (~5%) and FS (~15%) compared to vehicle-treated controls in both sexes. In addition, stroke volume was decreased significantly (p<0.05) after sunitinib exposure at days 3, 6, 9, 12 and 21 in both male and female mice. miRNAs play an important role in regulating both cardiovascular functions and pathways related to TNF. Preliminary results of miRNA profiling in plasma showed that highest number of miRNAs were differentially expressed in male (71 miRNAs) and female mice (85 miRNAs) at day 9 and 6 after sunitinib treatment, respectively when their LVEF and FS decreased significantly. Interestingly, miR-140-5p, one of the miRNAs involved in cardiomyocyte apoptosis, is consistently down-regulated at days 3, 6, 9, 12, and 21 in both sexes. These results suggest that this mouse model could be a valuable tool to uncover the mechanisms related to sunitinib-induced cardiotoxicity and identify early biomarkers for future clinical use.

**Mechanisms of Cadmium-Induced Aberrant Differentiation of Human Embryonic Stem Cells to Cardiomyocytes and Cardiac Organoid Formation Mimicking Heart Development**

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Cadmium (Cd) is a widespread environmental contaminant. Human exposure to Cd occurs mainly through ingestion of contaminated food or water. Cd exposure is associated with cardiovascular diseases, and maternal exposure to Cd is a significant risk factor for congenital heart disease (CHD). However, the molecular mechanisms associated with Cd-induced CHD are not well-defined. Embryonic stem cells (ESC) offer an excellent opportunity for studying the mechanisms of developmental toxicity. 3D aggregates of ESCs, called embryoid bodies (EBs), can be generated by treatment with thalidomide and exposure to Cd. EBs can be used as a model for embryonic heart development. In the present study, we developed a new model for the study of Cd-induced cardiotoxicity using EBs. To mimic the cardiac lineage, EBs were cultured in the presence of Activin A and Noggin. After 7 days of differentiation, EBs were treated with Cd at different concentrations. EBs were analyzed for changes in gene expression using qPCR and Western blot analysis. The results showed that Cd treatment downregulated the expression of genes related to cardiac differentiation and function. These findings suggest that Cd exposure during early development may induce circulatory failure. In the cardiac transcriptome analysis, analyses of KEGG pathways related to cardiac muscle contraction showed the dysregulation of genes related to Ca²⁺ transport and muscle filament sliding. As a result of quantitative RT-PCR, the gene expression levels of myosin receptor 2 (RYR2), myosin regulatory light chain 2 (MYL2), and myosin light chain 3 (MYL3) were decreased by Cd exposure. Transcription factor enrichment analysis activated NFKB1, SP1, SP3, SMA4D, GLI1, and CTNNB1 by Cd exposure as critical molecular initiating events. Peroxisome proliferator-activated receptor gamma (PPARγ) was also enriched as a relevant transcription factor. These results indicate that Cd exposure to chicken embryos may activate several transcription factors, including PPARγ, and alter the expression of genes related to the myocardial contraction, consequently leading to decreased heart rate and cardiac hypertrophy.

**Potential Cardioprotective Effects of Psilocybe natalensis Magic Mushroom Extracts on Angiotensin II-Induced Hypertrophy and Oxidative Stress in H9C2 Cardiomyocytes**

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Depression is a burden to societies. Psilocybin-containing mushrooms commonly known as magic mushrooms have been used since ancient and recent times for treatment of depression and mind healing. Their safety use in cardiovascular disease such as heart failure is not known and may pose as a risk. Cardiac hypertrophy is an independent risk factor for heart failure morbidity and mortality. Angiotensin II (AngII) triggers oxidative stress accumulation and plays a crucial role in the pathogenesis of cardiac hypertrophy. Our previous studies showed that Psilocybe natalensis, one of the well-known psilocybin mushrooms that grow in South Africa, has antioxidant and anti-inflammatory effects. We hypothesise that the mushroom extracts will not aggravate the AngII-induced hypertrophy condition. This study aimed at investigating safety of Psilocybe natalensis mushroom extracts on AngII-induced hypertrophy and oxidative stress on H9C2 cardiomyoblast cells. The mushrooms were grown and extracted with cold water, hot-boiling water and 70% ethanol. Cytotoxicity of the extracts was tested using tetrazolium-based assay. Cardiomyocytes were serum-starved for 18 hours, induced with 10 µM AngII and then treated with the extracts (25 µg/mL). Mitochondrial activity, cell size measurements, atrial natriuretic peptide levels, nitric oxide and intracellular reactive oxygen species productions (ROS) were measured. Osartan and Nw-nitro-L-arginine methyl ester were used as positive controls. The results showed that the extracts were not toxic on cardiomyocytes in the order ethanol>hot-water>cold-water. The extracts reduced AngII-induced hypertrophy indices and intracellular ROS levels. The effects were more pronounced with the ethanol and hot-water extracts. Extracts also reversed the AngII-induced cell death by increasing mitochondrial activity as indicated by cell viability. The study proposed that Psilocybe natalensis mushroom extracts were safe at the concentration used in the study, and did not worsen the Ang II-induced hypertrophy and oxidative stress in the stimulated cells. Study support safe medicinal use of the mushroom under control and cautioned higher concentration.

**Metallothionein Rescues Diabetic Cardiomyopathy in Akt2-Knockout Mice Probably via Activating ERK-Mediated Pathways**

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To develop an efficient and safe approach to prevent diabetic cardiomyopathy (DCM), a major cause of diabetic patient mortality, we have explored and confirmed that zinc supplementation prevents DCM via inducing cardiac metallothionein (MT) in type 1 and type 2 diabetes mouse models. Then we showed that MT prevents DCM via not only its anti-oxidative stress action, but...
also its insulin sensitizing function by increasing Akt2 phosphorylation and associated glucose metabolic pathways. This finding prompt us to further explore whether MT prevents DCM fully dependent on Akt2-mediated glucose metabolism pathways. We thus used mice with either global Akt2 knock-out (Akt2-KO), cardiomyocyte-specific overexpressing MT gene (MT-TG) or both (MT-TG/Akt2-KO). Experimental results confirmed the important role of Akt2 in maintaining glucose metabolism since Akt2-KO mice exhibit a type 2 diabetes phenotype and develop DCM (cardiac remodeling and dysfunction) along with cardiac inflammation and fibrosis. The expression of several molecules that involve in regulating glucose metabolic pathways is significantly reduced; however, Akt2-gene-deletion-associated DCM was unexpectedly almost fully prevented by overexpressing MT in Q mice. In addition, the cardiac protection by MT in MT-TG/Akt2-KO mice was accompanied by normally preserved the glucose metabolism, such as glycogen synthesis and glycolysis. These results suggest that MT regulates the glucose metabolism pathways not only by regulating Akt2, but also by other pathways that can somehow regulate Akt2-downstream signaling pathways in the absence of Akt2. We further found increased phosphorylation of ERK1/2 in the heart of MT-TG/Akt2-KO mice compared to those of Akt2-KO mice. Given that several studies have reported the important role of ERK1/2 in mediating Akt2 downstream glucose metabolism pathways, we assumed that the protection against Akt2 deletion by overexpressing MT may be associated with the ERK pathway.

### 2235 Chronic Cardiotoxic Effects of Kinase Inhibitors and Anthracyclines on Human iPSC-Derived Cardiomyocytes


In pre-clinical drug development, cardiac contraction analysis of potential drug candidates is one of the crucial steps to ensure a successful and reliable transition to clinical stages. The use of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) partly due to the inability of common cell-based assays to analyze cellular behavior reliably over prolonged periods of time. The aim of this study was to evaluate the applicability of hiPSC-CM contractility measurements for chronic toxicological assessment using the high-throughput FLEXcyte 96 system. We selected 15 kinase inhibitors and 3 anthracyclines with well-known cardiotoxic profiles to evaluate the reproducibility of clinical data. Cells from commercial sources were cultured on freely-swinging, ultra-thin and hyperelastic silicone membranes. Rhythmic contraction of the hiPSC-CMs resulted in dynamic deflection changes quantified by means of capacitive distance sensing. The resulting beat patterns were analyzed for essential inotropic parameters including amplitude, frequency, slopes of contraction and relaxation, area under curve and arrhythmic events. For the assessment of chronic compound effects, inotropic properties of the cells were recorded for five days. Compounds considered as clinically-cardio-safe showed negative inotropic effects only at micromolar concentrations, while compounds with demonstrated cardiotoxic profiles showed both time and concentration dependent inotropic effects as well as arrhythmic events at nanomolar concentrations. We conclude that our results indicate that the combination of hiPSC-CMs and the FLEXcyte 96 technology allows for cardiotoxicological studies beyond the current perspectives from acute to chronic evaluation of cardiotoxic risk compounds.

### 2236 Vascular Transient Receptor Potential Ankyrin-1 (TRPA1) and Acrolein: A Potent Agonist with Physiological and Toxicological Actions

**L. Jin**, **D. J. Conklin**, University of Louisville, Louisville, KY. Acrolein, an electrophilic α,β-unsaturated aldehyde, is present in foods and beverages, and is a product of incomplete combustion, and thus, acrolein reaches high ppm levels in smoke of cigarettes and structural fires. Exposure to acrolein has been linked to cardiopulmonary toxicity and cardiovascular disease. The hypothesis of this study is the direct effects of acrolein in isolated murine blood vessels (aorta and superior mesenteric artery, SMA) are dependent on the transient receptor potential ankyrin-1 (TRPA1) channel. Isolated aorta and SMA were exposed to increasing levels of acrolein using isometric myography. Acrolein-induced concentration-dependent (0.01-100 μM) relaxations in phenylephrine (PE) precontracted aorta (-74.6±7.5%) and SMA (-89.5±1.2%) of male and female mice. Because the SMA was both more responsive (% relaxation) and sensitive (>20x) to acrolein than aorta (SMA EC<sub>50</sub>: 8.8±0.2 μM; aorta EC<sub>50</sub>: 29.4±4.4 μM), the mechanisms of relaxation were studied in SMA. The acrolein-induced relaxation at low μM concentration was inhibited significantly by: 1) mechanically-impaired endothelium; 2) Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME); 3) guanonyl cyclase (GC) inhibitor (ODQ); and, 4) TRPA1 antagonist (A967079). Consistent with endothelium- and TRPA1-dependent mechanisms of relaxation, TRPA1 was localized in the SMA endothelium by immunofluorescence yet positive staining was also observed in smooth muscle layer. Compared with other known TRPA1 agonists, including cinnamaldehyde crotonaldehyde and allyl isothiocyanate (AITC), acrolein is a more potent vasorelaxant. Yet acrolein induced vasorelaxation and toxic responses in SMA at higher μM concentrations (similar to crotonaldehyde), and these responses were independent of TRPA1. Acrolein-induced relaxation depends on a functional endothelium and TRPA1, whereas vasoreactivity is enhanced by endothelium dysfunction. Thus, acrolein is both vasoactive (physiologically relevant) and vasotoxic (toxicologically relevant) in the SMA. The study also suggests that acrolein has been implicated in cardiopulmonary injury associated with exposure to high levels of acrolein, inhibiting TRPA1 systemically may interfere with sensitive vasoregulatory processes. Thus, more research is needed to understand and appreciate the contribution of TRPA1 to vascular control mechanisms.

### 2237 Zinc Supplementation Attenuates Cardiac Hypertrophy in High Fat Diet-Induced Obese Mice via Metallothionein


Obesity increases the risk of metabolic disorders such as insulin resistance, dyslipidemia, type 2 diabetes, and cardiovascular disease, and mortality. In obesity mouse model induced by high-fat diet (HFD), we found the increased insulin resistance and cardiac pathology including cardiac inflammation and hypertrophy, while zinc (Zn) supplementation prevented these detrimental changes along with increased metallothionein (MT) expression. Deletion of MT expression blocked the protection by zinc from palmitate-induced cardiac myocyte hypertrophy and inflammation in vitro. Here we further defined whether MT is essential for Zn prevention of HFD-induced cardiac hypertrophy. MT knockout (MT-KO) and 129S wild-type mice were fed normal diet (ND, 10%kcal fat) or HFD (60%kcal fat) from 8 weeks to 26 weeks, containing normal Zn (20 mg Zn/4057kcal) or supplemental (55, 90 mg Zn/4057kcal) Zn levels. HFD induced a time-dependent obesity in both WT and MT-KO groups, indicated by increased body weight and white adipose tissue, accompanied with insulin resistance in 18 weeks. At 26 weeks, glucose intolerance test showed ZS treatment slightly decreased insulin resistance in WT obese mice, but not in MT-KO obese mice. Compared to ND, HFD led to cardiac hypertrophy in MT-KO mice, not in WT mice. MT knockout increased cardiac hypertrophy and dysfunction in HFD-induced obesity group, illustrated by augmented heart weight (HW) and HW to tibia length ratio, interventricular septum thickness, left ventricular posterior wall, decreased ejection fraction and fractional shortening evaluated by echocardiography. ZS treatment did not improve cardiac hypertrophy and cardiac dysfunction in MT-KO obese mice compared to ZN MT-KO obese mice. In conclusion, MT knockout exacerbated cardiac remodeling and myocardial dysfunction associated with HFD-induced obesity and zinc could improve cardiac function under obesity context via MT.

### 2238 The Effects of Whole-Life, Low-Dose Cadmium Exposure on High Fat Diet-Induced Cardiac Pathologies in Female Mice

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Both cadmium (Cd) exposure and obesity increase the risk of cardiovascular disease. We have shown post-weaning feeding of high-fat diet (HFD, 60% kcal fat) induces cardiac hypertrophy in mice and exposure to low-dose Cd for four weeks induces cardiac remodeling. Therefore, we wanted to determine whether whole-life exposure to environmentally-relevant, low-dose Cd affects heart structure and function, and whether the susceptibility of their female offspring to post-weaning feeding of HFD-induced cardiac pathologies is increased. Therefore, adult male and female C57BL/6J mice fed normal diets (ND, 10% kcal fat) were exposed to 0 or 5 ppm Cd-containing drinking water starting one week before mating, continuing through weaning of offspring. After weaning, female offspring were fed ND or HFD and continued on the same drinking water regimen as their parents for 24 weeks. Consequently, there were four experimental groups of female offspring: Control, HFD, Cd,
2239  Cardiovascular Toxicity of Trichloroethylene

Trichloroethylene (TCE) is a chlorinated solvent present in air, water and soil. In the United States, TCE has been detected in >800 Superfund sites. It is a human and rodent carcinogen, and TCE also has adverse health effects on central nervous system, liver and immune system. Although gestational TCE exposure is reported to induce fetal heart defects, chronic TCE exposure is known about chronic cardiovascular toxicity of TCE. Thus, sub-clinical markers of cardiovascular toxicity were measured in adult female and male C57BL/6J mice exposed to drinking water alone or with TCE (0.5 mg/ml) for 1 year. In female mice, TCE exposure significantly slowed weight gain (22%, P<0.05), decreased percent fat (20%, P<0.05), and depressed mean and diastolic arterial blood pressure; however, cardiac and aortic function, platelet activation, and glucose tolerance were unaffected. In contrast, TCE-treated male mice had a significant decrease in cardiac fractional shortening (12%, P<0.05; by echocardiography) and enhanced aortic sensitivity to nitric oxide donor, yet had unchanged arterial pressures. Flow cytometric analyses detected significant increases in both circulating platelet-monoocyte and platelet-leukocyte cppy adducts (>30%, P<0.05) as well as a significant increase (>40%, P<0.05) in blood endothelial microparticles, activated endothelial microparticles, endothelial progenitor microparticles and platelet microparticles. TCE-exposed male mice also displayed increased glucose concentration. TCE exposure did not affect the levels of bone marrow stem cells, circulating endothelial progenitor cells and immune cells in male or female mice. Together, these data suggest that chronic TCE exposure can induce sex dependent changes in cardiovascular dysfunction and platelet activation.

2240  Preclinical Assessment of Antiviral Drugs Using Human iPSC-Derived Cardiomyocytes

Anti-viral drugs are an important part of the arsenal of therapies used to treat symptoms of viral infections such as Ebola, the common flu, and acquired immune deficiency syndrome virus. In the course of the development of any pharmaceutical drug, including anti-viral drugs, it is imperative to understand the cardiac safety profile. In vitro evaluation of cardiotoxicity at the cellular level may provide key insights toward better prediction and mitigation strategies for pharmaceutical drugs in development. Here, we utilized human iPSC-derived cardiomyocytes in conjunction with a real-time cell analysis system, which combines impedance and field potential measurements to evaluate the electrophysiological, contractile, and structural impact of anti-virals alone or in combination with other drugs. Cells were exposed to the test antiviral drugs for 5 days and monitored for cellular changes in real-time. Our data demonstrate that 1) favipiravir, an influenza drug and sofosbuvir, an anti-hepatitis C virus (HCV) drug, had marginal effects on cell viability, contractile and electrical activities of hiPSC cardiomyocytes up to 30-fold Cmax suggesting that they were safe in vitro; 2) Remdesivir, however, showed a dose and time-dependent reduction in viability and suppression of contractility demonstrated by decreased beating amplitude and slowing rate. The irregular induced cardiac remodeling and dysfunction was observed 3 days after exposure to 5 μM (Cmax) remdesivir and there were no significant changes in field potential duration (FPD) observed at all tested concentrations. At higher doses, 15 μM and above remdesivir immediately caused cell quiescence in addition to causing reduced field potential amplitude and slowing rate. The irregular induced cardiac remodeling and dysfunction was observed 3 days after exposure to 5 μM (Cmax) remdesivir and there were no significant changes in field potential duration (FPD) observed at all tested concentrations. At higher doses, 15 μM and above remdesivir immediately caused cell quiescence in addition to causing reduced field potential amplitude and slowing rate. 3) While sofosbuvir appeared safe by itself, in combination with amiodarone resulted in a profound increase in beating rate and decrease in beating amplitude as well as a decrease in viability. Taken together, human iPSC cardiomyocytes in conjunction with the real-time cell analysis system provides a comprehensive cardiac safety assessment of antiviral drugs, and are amenable for the identification and evaluation of cardiac drug-drug interaction.

2241  Mechanistic Investigation of Delayed Cardiovascular Effects of an IRAK4 Inhibitor in Monkeys
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An IL-1R kinase inhibitor (BMS-986323) induced multifocal premature ventricular contractions (PVCs) and non-sustained ventricular tachycardia in a repeat-dose telemetry study in cynomolgus monkeys. Arrhythmias occurred after 2-4 days of dosing, were reproducible, and were not associated with cardiac levography, electrocardiography, elevations in cardiac troponin, or effects on cardiac repolarization or conduction. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) were used to determine whether arrhythmias were target-related and to explore mechanism. Multi-electrode array (MEA) electrophysiology and fluorescence detection for intracellular calcium were used to assess effects of BMS-986323, its metabolites, and structural analogs with and without kinase activity for up to 96 hours. MEA endpoints included spontaneous beat rate, inter-pulse interval CoV (surrogate for beat regularity), field potential afterdepolarizations (trigger for ectopic beats, PVCs), conduction velocity and field potential duration (FPD, reported to indicate conduction delay, QT interval, QT, QT2 interval). Media supernatants were sampled at 24, 48 and 96 hours for measurement of troponin I, brain natriuretic peptide, fatty acid binding protein 3, and growth/differentiation factor 15. At free concentrations that produced arrhythmias in monkeys, BMS-986323 did not produce arrhythmic signals 2 hours after treatment but induced early afterdepolarization (EAD)-like waveforms at ≥24 hours. BMS-986323 had no significant effect on FPD or conduction, or on any of the cardiotoxicity biomarkers. Absence of effects of metabolites suggests that arrhythmias are due to a delayed effect of parent rather than delayed formation of a metabolite. An inactive structural analog also induced EADs at ≥24 hours and a structurally divergent inhibitor of the same kinaase did not further implicating an off-target effect. Because BMS-986323 had no effect on several causes of triggered activity in hiPSC-CMs or in monkeys including repolarization or conduction delay or cardiotoxicity, effects on another potential mechanism, intracellular calcium dysregulation, were explored. BMS-986323 prolonged intracellular calcium transients and produced ‘shoulders’ on the decay phase, suggesting that the mechanism for arrhythmias may involve calcium dysregulation. Nifedipine (10 mM) blocked EADs observed on MEA and inhibited the changes in intracellular calcium. These studies in hiPSC-CMs provided insights into target involvement, mechanism, and potential treatment for arrhythmias initially detected in repeat-dose monkey studies with BMS-986323.
Waterpipe smoke (WPS), a type of tobacco smoke, is currently rising in popularity with both men and women in the United States. Despite this alarming trend and waterpipe’s centuries-long history, little is known about the long-term impact of chronic waterpipe smoke (WPS) on the development of atherosclerosis. Apolipoprotein E deficient (ApoE-/-) mice have been shown to exhibit increased atherosclerotic plaque development in the brachiocephalic artery and the aortic arch. The development of atherosclerotic plaques is exacerbated by inhalation of particulate matter from many sources, including tobacco smoke. Therefore, it was postulated that WPS, even though it is bubbled through water and is thought to be ‘cleaner’ may also promote atherosclerotic plaque progression. ApoE-/- male and female mice were exposed to either diluted WPS (46.46 ± 24.82 mg/m³) or purified air via nose-only inhalation for 2 hours/day, 4 days/week, for 5 months. The brachiocephalic, left subclavian and left carotid arteries as well as aortic arch of each animal were excised and transversely sectioned and stained for analysis of intima-media thickness and area. Animals exposed to WPS exhibited sex-dependent differences in the intima-media thickness and area compared to air controls. Analyses indicated a greater degree of plaque formation in the ascending and descending aortas of WPS-exposed males, in contrast to thinner intima-media of aortas in females when compared to controls. The small arteries indicated opposing trends, with thinner intima-media areas in WPS-exposed males. Overall, these results indicate a divergence of atherosclerotic responses to long-term WPS in male and female ApoE-/- mice. The current study indicates that WPS influences the progression of cardiovascular disease in a sex-dependent manner in susceptible individuals.
methemoglobin formation, disproportionation reaction, intravascular residue time, partitioning between blood components, and blood-brain barrier penetration. As a good redox system, DMTS was found to be able to oxidize hemoglobin to methemoglobin. During the DMTS disproportionation reaction, DMDS, DM4AS, and DM5SS were all identified to be products. The intravascular residue time study resulted in a half-life of 36 minutes. As a small lipophilic molecule, DMTS was found to be oxidized by the liver and accumulated in the red blood cell (RBC). The ability of RBCs, plasma, and albumin to bind DMTS was also investigated, resulting in the binding—elimination rate order as follows from a shorter half-life to a longer half-life: whole blood, plasma, RBC, then albumin. These results reveal the importance of DMTS elimination from the bloodstream where RBC and albumin can be considered as the main sink of DMTS. The blood brain barrier penetration was determined by the in vitro parallel artificial membrane permeability assay (clearance determination) and the in vivo organ distribution study. The ability of the antidote molecule to permeate this membrane is important since the brain is one of the major target organs affected by OP intoxication, alongside the heart. This research was supported by the CounterACT Program, National Institute of Health Office of the Director, and the NIAID, Inter Agency Agreement Number Y1-OD-1561-01/A120-B.P2011-01, and the USAMRICD under the auspices of the USAROSSP Contract No. W911NF-11-D-0001 and the Robert A. Welch Foundation (x-001) at Sam Houston State University, Huntsville, TX.

In Vivo Brain Imaging of Microglia following Nerve Agent Exposure Utilizing Two-Photon Microscopy in Mice

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Organophosphate (OP) cholinesterase inhibitors cause excessive excitatory signaling in the central nervous system that can result in persistent seizures and prolonged brain damage. A robust neuroinflammatory response is associated with seizure activity and may continue after seizure termination, leading to an increased risk for progressive neurodegeneration. This study aimed to evaluate (1) the neuroinflammatory response that follows after seizure termination, characterized by the activation of microglia, and (2) whether the cyclin-dependent kinase (CDK) inhibitor CR8 is effective in mitigating this response. Microglia were monitored in a 3 mm by 3 mm region of the cortex in mice, before and after OP exposure. This was achieved by utilizing a two-photon microscope and harness system to track changes occurring 500 µm into the brain deep in the cortex of the brain via a surgically implanted cranial window. Transgenic mice were enabled for the observation of microglia (B6.129P-Cx3cr1tm1Litt/J) via green fluorescent protein (GFP). The mice were imaged 1 week prior to OP exposure (sarin, 256 µg/kg; SC) and again at 24-, 48-, and 72-hour time points. The oxime Hi-6 (50 mg/kg; IP) was given 30 min prior to OP exposure, and atropine (1 mg/kg; SC) was given immediately following OP exposure to increase survival. The machine-learning trainer by Arivis Vision4D modular software was used to obtain microglial counts within the Object Mask Analysis operation, and a maximum intensity projection was performed to analyze fluorescent intensity. The images taken were processed as distinct clusters and migration of the observed microglia, characterized by an increase in size, fluorescent intensity, and number. The results suggest that nerve agent casualties are still at risk for progressive neurodegeneration from the continued inflammatory processes occurring after successful seizure termination. Animals treated with CR8 demonstrated inhibited microglial proliferation, represented by reduced cell counts and lower fluorescent intensities. A resting state cell morphology was observed in CR8-treated animals, in comparison to the activated cell morphology seen in non-treated animals. Collectively, these results suggest CR8 has the potential to aid in neuroprotection following OP-induced seizures.

Immediate-Early Gene Expression May Help Explain Hippocampal Neuroprotection of a Novel Phenoxyalkyl Pyridinium Oxime Antidote to Organophosphates

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Past assassinations and terrorist attacks demonstrate the need for a more effective antidote against nerve agents and other organophosphates (OP) which cause brain damage through inhibition of acetylcholinesterase (AChE). Our lab has designed novel phenoxyalkyl pyridinium oximes (US. pat. 9,227,937) that demonstrate the ability to cross the blood-brain barrier and attenuate hippocampal damage in a rat model (Dail et al, 2019 Tox Sci 169:465). This project examined whether expression changes in immediate-early genes may help explain this protection. Sprague Dawley-derived rats were subcutaneously (SC) given either the vehicle (DMSO) or a high sublethal dosage (0.325 mg/kg) of the sarin surrogate, nitrophenyl isopropyl methylphosphonate (NIMP). One hour later peak inhibition (about 80%) of brain AChE and signs of hypercholinergic toxicity, including seizure-like behavior, occur. At this point, rats were intramuscularly (IM) given 146 µmoles/kg of 2-PAM or novel Oxime 20 in Multisol (48.5% H2O, 40% propylene glycol, 10% ethanol, 1.5% benzyl alcohol) or Multisol alone. The hippocampus was dissected, snap frozen in liquid nitrogen, and stored at -80°C. RNA was isolated with the Qiagen RNAeasy Plus Mini Kit and examined by duplex qPCR on a Stratagene MX3005 qPCR system using IDT PrimeTime qPCR Assays and Gene Expression Master Mixes. Expression of genes involved in brain repair (Bdnf), astrocyte damage (Gfap), initiation of neuronal regeneration (Fos), and apoptosis control (Jdp2, Bcl2l1, Bcl2l11) were normalized to the housekeeping gene RPLP1, then compared across the treatment groups; NIMP alone, oxime alone, NIMP followed by oxime, and vehicle. Gfap, Jdp2, and Bcl2l11 showed no statistically significant changes in any treatment group. Fos and Bdnf expression were not significantly altered from vehicle levels by NIMP/Oxime 20, but were significantly decreased and increased, respectively, by NIMP/2-PAM treatment. Bcl2l11 expression was significantly decreased by NIMP. Although this was reversed by both oximes, Oxime 20 stimulated expression more than 2-PAM and returned it to a level closer to that of the vehicle. Since Bdnf, Fos and Bcl2l11 are all involved in aspects of neuronal survival, their Oxime 20 associated expression could explain the observed attenuation of NIMP-related hippocampal damage. Support: NIH U01NS101727.

Delayed Treatment with the Cyclin-Dependent Kinase Inhibitor CR8 Provides Neuroprotection following Nerve Agent-Induced Seizures

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Organophosphates (OP) act primarily upon acetylcholinesterase in the central nervous system, initiating a cholinergic crisis. This excessive excitatory state can lead to seizures which are defined as status epilepticus (SE) when maintained for five minutes or more. SE resulting in neuroinflammation can lead to severe brain damage and progressive neurodegeneration. Significant work has been put into mediating the initial occurrence of SE, but few studies have examined in the neuroanatomical loss in the time following SE. Previously we have shown that progressive neurodegeneration occurs after SE termination and can be mediated via the cyclin-dependent kinase (CDK) inhibitor CR8. Here we evaluated in what timeframe administration of CR8 was effective in mitigating neuronal damage and investigated Ibuprofen, a readily available over-the-counter drug with similar impacts on the cell cycle, to achieve the same neuroprotective effect. Mice were surgically prepared two weeks prior to the experiment with electrodes to record brain activity. The oxime Hi-6 (50 mg/kg; IP) was given prior to nerve agent exposure to increase survival without finding SE onset. Mice were exposed to the nerve agent sarin to elicit SE (256 µg/kg; SC; control animals received an equivalent volume of saline) followed by the anti-muscarinic atropine (1 mg/kg; SC). The CDK inhibitor CR8 (5 mg/kg, IP) was administered at various time points and intervals following SE onset; alternately some animals received the same schedule of ibuprofen out to 72 hrs following exposure significantly reduced the number of activated microglia and degenerating neurons in the hippocampus. In comparison to CR8, daily ibuprofen was less effective at providing neuroprotection following SE termination. Finally, this study also found that CR8 reduced neuronal damage even in cases of unterminated SE. Collectively, the results suggest that CR8 is an effective neuroprotective therapy following OP exposure.

A Comparison of In Vitro Reactivation Efficacy of Novel Substituted Phenoxyalkyl Pyridinium Oximes for Organophosphate-Inhibited Human and Rat Acetylcholinesterase

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In vitro tests were conducted to compare the efficacy of 4 novel oximes and pralidoxime (2-PAM) for human and rat acetylcholinesterase (AChE). The novel oximes were from a platform of substituted phenoxyalkyl pyridinium oximes (US Patent 9,227,937). The organophosphates (OPs) tested were a novel oxime 20 as well as pralidoxime (2-PAM) for human and rat acetylcholinesterase (AChE). The oxime Hi-6 (50 mg/kg; IP) was given prior to nerve agent exposure to increase survival without finding SE onset. Mice were exposed to the nerve agent sarin to elicit SE (256 µg/kg; SC; control animals received an equivalent volume of saline) followed by the anti-muscarinic atropine (1 mg/kg; SC). The CDK inhibitor CR8 (5 mg/kg, IP) was administered at various time points and intervals following SE onset; alternately some animals received the same schedule of ibuprofen out to 72 hrs following exposure significantly reduced the number of activated microglia and degenerating neurons in the hippocampus. In comparison to CR8, daily ibuprofen was less effective at providing neuroprotection following SE termination. Finally, this study also found that CR8 reduced neuronal damage even in cases of unterminated SE. Collectively, the results suggest that CR8 is an effective neuroprotective therapy following OP exposure.
active metabolite of the insecticide parathion, paraoxon (PXN). The source of AChE was erythrocyte "ghost" preparations from both species; these are composed of washed cell membranes and can be used as a model of AChE in the nervous system since the AChE is the same gene product in both tissues. The human blood samples were purchased from a commercial vendor. Rat blood samples were collected from Sprague Dawley rats. Preparation involved centrifugal separation of the erythrocytes, lysis of the cells and three washes to remove hemoglobin. The reactivation potential of the approved antidote 2-PAM and novel oximes 1, 15, 20, and 55 against NIMP and PXN was determined. Concentrations of NIMP (3.16 µM) and PXN (10 µM) were selected to yield about 80% AChE inhibition during a 15-minute incubation period, followed by oxime (100 µM) for a 30-minute incubation of reactivation. AChE activity was assayed by a modified Ellman method, 1 mM acetylthiocholine as a substrate and DTNB as the chromogen, with three replications to calculate the inhibition and reactivation compared to solvent controls. For NIMP, novel oxime reactivation was similar or higher for all 5 oximes for the human (82-92%) than for the rat (72-88%). Additionally, reactivation was higher for all novel oximes than for 2-PAM with NIMP in rat and human samples. For PXN also, novel oxime reactivation was similar or higher for all 5 oximes for the human (21-64%) than the rat (13-64%). With PXN, reactivation with 2-PAM was higher than with the novel oximes. These data indicate that the novel oximes show reactivation efficacy toward OP-inhibited AChE from humans at least as good or higher than for rats, and that the efficacy results that are currently being obtained in rat experiments should be representative of reactivation efficacy that would occur in humans. Supported by NIH:U01 NS107127.

2252 Pharmacokinetics of Three Novel Pyridinium Aldoxyme Acetylcholinesterase Reactivators in Female Rats

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Organophosphates compounds (OP) are some of the most potent known acetylcholinesterase (AChE) toxins. OP inhibition of AChE leads to over-activation of the nervous system, and can result in death. Treatment of OP intoxication with atropine and the AChE reactivator 2-pyridinium aldoxime (2-PAM) is currently the US’s FDA-approved therapeutic intervention strategy. However, the small, highly polar nature of 2-PAM limits its ability to traverse the blood-brain barrier to reactivate inhibited AChE in the brain. Novel substituted phenoxylaryl pyridinium aldoximes (US patent: 9,227,937) have been designed with enhanced lipophilicity to facilitate their permeation into the central nervous system (CNS). Previous studies by our group have provided histological evidence of in vivo neuroprotective effects by MSU 20 and 55, but not MSU 15. In this study, the pharmacokinetic profiles of these 3 were determined using a LC-MS/MS method that we validated. Female rats received either a single 50 mg/kg intramuscular (IM) or a 5 mg/kg intravenous (IV) dosage of each oxime. Whole brains were collected from the IM treated group at 2 h post-dose to assess the accumulation of oximes within the CNS. Plasma samples were collected at 5, 15, 30, and 45 min, and 1, 2, 4, 12, and 24 h post-dosing. Oxime concentrations in the brain at 2 h followed the trend: MSU 20 (135 ng/g brain) > MSU 15 (126 ng/g brain), whereas MSU 55 was detectable but, below the limits of quantitation. The observed plasma half-lives (T1/2) following IM administration followed the trend: MSU 55 (0.45 h) > MSU 15 (0.15 h) = MSU 20 (0.13 h). Maximal plasma concentrations (Cmax) were achieved for MSU 15 at 0.78 h and for MSU 20 and 55 at 0.08 h after IM injection followed the opposite trend of the half-lives with MSU 15 (2977 ng/mL) > MSU 20 (2368 ng/mL) > MSU 55 (1040 ng/mL). After IV injection, Cmax achieved at 0.08 h, followed the trend MSU 55 (3108 ng/mL) > MSU 15 (2935 ng/mL) > MSU 20 (1852 ng/mL). Overall, these pharmacokinetic data (T1/2) and similar trends to previously reported data in male rats indicating that the neuroprotective ability of MSU 20 and 55 was probably related to their rapid absorption into the blood stream and extended levels in the blood stream, allowing extended entry into the brain. The data also indicate that there were few sex differences in pharmacokinetic behavior. Support: NIH U01NS107127.

2253 Novel Pyridinium Oximes in Combination with 2-PAM or Another Novel Oxime: Potentiate Survival following Organophosphate (OP) Exposure in Rats

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Acetylcholinesterase (AChE) inhibition by OPs, including nerve agents and some insecticides, results in overstimulation of the nervous system and may lead to death. Traditional therapies for OP exposures include atropine, and an oxime to reactivate inhibited AChE, 2-PAM in the US. Although 2-PAM is an effective AChE reactivator and can increase survival following OP intoxication, it has limited ability to cross the blood-brain barrier (BBB) and reactivate inhibited AChE in the central nervous system. Novel pyridinium oximes (US patent: 9,227,937) synthesized to increase lipophilicity and BBB penetration have been shown to reactivate OP inhibited AChE, attenuate seizure-like behavior, decrease neuropathology and increase survival in rats. The most efficacious of these oximes were tested in binary mixtures with 2-PAM or one of the other novel oximes in rats administered lethal doses of a sarin surrogate or paraoxon. A sarin surrogate, nitrophenyl isopropyl methylphosphonate, NIMP (0.6 mg/kg) or paraoxon (PXN, 0.8 mg/kg) were administered SC in rats followed by atropine IM (0.65 mg/kg) and binary mixtures of 2-PAM and novel oximes IM (146 µmoles/kg each) at the onset of seizure-like behavior. Novel oximes in combination with 2-PAM yielded 53-87% and 67-93% survival, while combinations of novel oximes yielded 47-87% and 47-60% survival for NIMP and PXN, respectively, in male rats. Similarly in female rats matched for estrus cycle, novel oximes in combination with 2-PAM yielded 67-83% and 73-87% survival for NIMP, and PXN, respectively, while combinations of novel oximes yielded 47-67% survival for NIMP and 47-60% for PXN. Combinations of oximes yielded equivalent or greater survival than a single oxime and attenuated seizure-like behavior. In addition, 24-hour survival was determined for mixtures of 2-PAM and novel oximes in guinea pigs (MRIGlobal) challenged with a LD50 of sarin. Guinea pigs were monitored and scored for signs of toxicity. Survival for mixtures of novel oxides and 2-PAM ranged from 63-87%. Toxic signs scores were lower for animals receiving mixtures of oximes. Preliminary safety studies were conducted on the novel oximes in binary mixtures with 2-PAM. Male rats were administered oxime mixtures and observed for 14 days. No signs of overt toxicity were observed. Results suggest these oximes have therapeutic potential for OP exposures. Support: NIH:U01NS107127.

2254 Sulfur Mustard-Induced Changes in the Epidermal Keratins in Mouse Skin

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Vesicant, sulfur mustard (2,3-dichloroethyl sulfide, SM), is a chemical warfare agent causes blistering and severe tissue damage. The characteristic cutaneous injury induced by SM in mouse ear skin including edema, erythema, microblistering, epidermal hyperplasia, accompanied by strong inflammation and leading to prolonged wound repair. Skin structured with multilayers of keratinocytes (KCs), serves as a protection barrier against toxic chemicals. Keratins (Ks), the major structural intermediate filaments proteins in KCs, may regulate skin wound healing. In normal skin epidermis, Ks express K5 in basal cells and K10 in the differentiated suprabasal layers. Upon skin injury, KCs, activated by inflammation, express K6 and K17 to promote KCs migration for wound repair. Dysregulation of activated epidermal KCs may result in barrier breach and lead to uncontrolled inflammation and skin diseases. In addition, studies showed the small proline-rich (Spr) family of cornified envelope (CE) precursor proteins protect from reactive oxygen species (ROS) damage in skin. The present study was focused on SM-induced changes in the composition of epidermal keratins and CE in mouse ear skin. Time course studies (1-7 days) of mouse ear skin exposing to a single dose (0.08 mg) of SM showed characteristic histopathology changes over time. Significant changes of K10, K6, K17, and Spr18 gene expression were observed in SM injured skin post exposure. Immunohistochemistry results showed the K6 and K17 staining was restricted to the skin appendages in the naïve control, whereas samples exposed to SM had increased expression over time in the suprabasal epithelium. The activated KCs highly expressed K6, K17, and Spr18 in the wound edge. In contrast, reduced expression of the differentiation marker, K10, indicated incompletely differentiated epithelium, suggesting weakened barrier protection in skin damaged by SM. Multiplex immunofluorescent studies showed heterogeneous coexpression of K5/ K6/K10 and K5/K10/Spr18 in the hyperplasia behind the leading edge, suggesting hyperactivated KCs in the SM injured epidermis. Overall, K10, K6, K17, and Spr18 have value as skin wound and repair markers of SM induced injury. Understanding the molecular mechanism of action of vesicant-induced skin injury and repair involved in epidermal keratinocytes may help to identify new targets of medical countermeasures to chemical vesicant skin injury. Supported by NIH grants E5005022 and AR053073.

Virtual 2021 SOT Annual Meeting and ToxExpo
Nitrogen mustard (NM) is a chemical warfare agent that causes acute lung injury which progresses to fibrosis. In the present studies, we assessed NM-induced lung injury in live animals using magnetic resonance imaging (MRI) and computed tomography (CT). Structural changes identified in the lung were then used to predict alterations in pulmonary mechanics. Male Wistar rats were imaged by MRI and CT one day prior to and then 28 d post intratracheal administration of PBS control or NM (0.125 mg/kg). Following imaging, rats were anesthetized, and lung function assessed at a positive end expiratory pressure (PEEP) of 3 cm H2O using a Scireq flexiVent® small animal ventilator. Forced oscillation measurements of lung function and quasi-static pressure volume loops were generated in triplicate. MRI images were used to characterize lung injury by quantifying opacities in the lung which represented tissue remodeling (i.e., fibrosis). Opacities present in MRI images from NM exposed animals were not present in control animals. CT images were used to assess changes in lung volume. Changes in lung volume relative to pre-exposure lung volumes were reduced in rats exposed to NM at 28 d (-1.3 x 10^-5 vs. 3.9 x 10^-5 Hounsfield units). Components from MRI and CT imaging analysis were then evaluated using a feed forward model to predict lung function. These predictions were correlated to measurements of lung function. Forward modeling was used to predict respiratory impedance, which calculates resistance (real in-phase component) and elastance (imaginary out-of-phase component) of the pressure-flow relationship over a range of frequencies. Measured and modeled real and imaginary impedances resulted in strong correlations for both control and NM treated rats (r²=0.96). These data demonstrate that MRI and CT imaging can be used to predict functional deficits in the lung following NM exposure, suggesting a potential approach for evaluating disease progression and therapeutics in live animals.

NIH AR055073, ES004738, ES029254, ES005022, and HL086621.

2255 Predicting Alterations in Lung Function Based on Structural Changes Identified in Live-Animal Imaging following Exposure of Rats to Nitrogen Mustard

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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 a robust inflammatory cell response, including activation of resident alveolar macrophages and an accumulation of bone marrow-derived monocyes in the lung, which develop into pro-inflammatory/cytotoxic M1 macrophages and anti-inflammatory/pro-fibrotic M2 macrophages. Farnesoid X receptor (FXR) is a bile-acid activated nuclear receptor that is highly expressed in liver and intestine; it has also been shown to exert anti-inflammatory activity. In previous studies we showed that expression of FXR, along with two of its targets, ApoA and ApoE, were upregulated in lung macrophages following NM exposure. In these studies, we analyzed the role of FXR in regulating macrophage accumulation and activation in the lung following NM exposure. We focused on male and female wild type (WT) and FXR-/- mice which were treated with PBS (control) or NM (0.08 mg/kg) via intratracheal instillation. Lung tissue and bronchoalveolar lavage (BAL) fluid were collected 3, 14, and 28 days later. NM caused histopathologic alterations in the lung including inflammatory cell infiltration, septal damage, and epithelial thickening, which were more prominent in FXR-/- mice relative to WT mice at all post exposure time points. This correlated with aggravated increases in BAL protein and cell content. Flow cytometric analysis revealed that both pro- and anti-inflammatory macrophages accumulated in the lung following NM exposure. Pro-inflammatory macrophages were most abundant in BAL 3 days post NM, a response more pronounced in FXR-/- mice. In male but not female mice, increases in proinflammatory macrophages persisted in the lung for at least 28 d post NM. In contrast, while anti-inflammatory macrophages also increased in the lung following NM administration, no differences were observed between WT and FXR-/- mice. These findings demonstrate that FXR modulates proinflammatory macrophage responses to NM in a sex-dependent manner and suggest a potential new target for the development of therapeutics to blunt mustard vesicant induced lung inflammation and injury. Supported by NIH AR055073, ES020721, ES004738 and ES005022.

2256 Oxidative Stress, Metabolic Dysfunction, and Apoptosis in Alveolar Epithelial Type II Cells following Exposure of Rats to Nitrogen Mustard


Nitrogen mustard (NM) is a cytotoxic vesicant known to target the lung, causing acute injury, oxidative stress and impaired function. Alveolar epithelial Type II (TII) cells generate surfactant proteins, which are important in lowering surface tension at the air-liquid interface thus preventing alveolar collapse at end-expiration. In the present studies, we determined if NM-induced dysregulation in lung function is a consequence of aberrant TII cells activity. Male Wistar rats (150-174 g; 8-10 weeks) were euthanized 3 days after intratracheal administration of NM (0.125 mg/kg) or PBS control. TII cells and bronchoalveolar lavage (BAL) fluid were collected 3 d later. NM exposure resulted in increases in the number of TII cells recovered from the lung; PCNA expression by these cells also increased, consistent with Type I alveolar epithelial cell toxicity. Expression of heme oxygenase (HO)-1 and nitrotyrosine, markers of oxidative and nitrosative stress, were upregulated in TII cells following NM exposure, suggesting a potential response more pronounced in TII cells from NM treated rats. Thyroid transcription factor (TTF-1) plays a role in regulating surfactant proteins. Following NM exposure, TTF-1 was significantly reduced in TII cells; this was associated with increases in surfactant protein (SP)-D levels and alterations in SP-D structure as reflected by S-nitrosylation of SP-D and the appearance of SP-D multimers. These data demonstrate that TII cells are directly targeted by NM, which may be important in altered pulmonary mechanics following exposure to mustard vesicants. Support: NIH AR055073, ES004738 and ES005022.

2257 Farnesoid X Receptor Regulates Immune Cell Activation and Recruitment to the Lung following Exposure of Mice to Nitrogen Mustard

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Nitrogen mustard (NM; bis(2-chloroethyl)methylamine) is a cytotoxic vesicant known to cause acute lung damage. In these studies, we developed mathematical models that allow prediction of NM-induced functional deficits in the lung based on structural alterations identified by magnetic resonance imaging (MRI) and computed tomography (CT). Male Wistar rats were exposed to PBS (CTL) or NM (0.125 mg/kg) via i.t. instillation. CT and MRI scans were performed at 28 d post exposure. Pulmonary function was assessed using a Scireq flexiVent at a positive end expiratory pressure (PEEP) of 3 cm H2O. Because of accuracy and minimal artifacts, MRI data was used to calculate total lung volume (V). As CT is capable of differentiating between regions of air and water within the lung, it was used as a direct measure of functional deficits within the tissue. Relative lung volume (V/VT-1) and CT data were used to summarize hyperinflation (I), normal lung, and consolidated lung tissue (B) using voxel density. Using these elements, a feed forward model was constructed to predict respiratory impedance (Z), a measure of airflow, with the equation: \( Z = (\alpha + \beta)/\omega \) where \( \omega = B/(I+iB) \). Calculated values of \( B \) from imaging data confirmed alterations in lung structure following NM exposure. A response more pronounced in FXR-/- mice. In male but not female mice, increases in proinflammatory macrophages persisted in the lung for at least 28 d post NM. In contrast, while anti-inflammatory macrophages also increased in the lung following NM administration, no differences were observed between WT and FXR-/- mice. These findings demonstrate that FXR modulates proinflammatory macrophage responses to NM in a sex-dependent manner and suggest a potential new target for the development of therapeutics to blunt mustard vesicant induced lung inflammation and injury. Supported by NIH AR055073, ES020721, ES004738 and ES005022.
Vesicating agents, including sulfur mustard (bis(2-chloroethyl) sulfide) and nitrogen mustard mechlorethamine (HN2, bis(2-chloroethyl) methyleneimine), are bifunctional alkylating agents that are highly reactive with cellular DNA and proteins in the skin causing extensive tissue damage and blistering. Amifostine (2-(3-aminopropyl) aminoethylphosphorothioate; AMF) is an FDA-approved thio phosphate produg which is activated in vivo to WR-1065, a cytoprotective thiol metabolite. Its actions are attributed to a sulfhydryl-containing polyamine-like structure that scavenges free radicals and participates in a wide variety of cellular processes including DNA repair. In the present studies, we evaluated the effects of AMF on HN2-induced toxicity in human keratinocytes. HN2 treatment caused time- and concentration-dependent DNA damage leading to a block in the S phase of the cell cycle in both primary human epidermal keratinocytes (HEK) and immortalized human keratinocytes (HaCat). This was associated with site-specific phosphorylation of DNA damage response (DDR) proteins including ATM (5198), ATR (T1989), CHK2 (T68), H2AX (S139), and p53 (S15). Phosphorylated DDR proteins were found to accumulate in DNA damage foci in keratinocytes. Flow cytometric analysis revealed that activation of DDR signaling was cell-cycle-dependent; a more pronounced increase in DDR phosphoproteins was evident in cells in S phase relative to cells in G1 and G2/M. Treatment of keratinocytes with AMF (4 mM) or WR-1065 (200 µM) 30 min after HN2 suppressed HN2-induced DNA damage and cell cycle arrest. AMF and WR-1065 also increased cell viability in HN2-treated cells. Western blotting showed that AMF selectively enhanced HN2-induced phosphorylation of ATM and CHK2 with no significant effects on ATR and CHK1, suggesting preferential stimulation of repair of double strand DNA breaks. Taken together, these data demonstrate that AMF can enhance DDR signaling and DNA repair processes and reduce HN2-induced toxicity in keratinocytes. AMF may be an effective countermeasure for mitigating vesicant-induced skin toxicity. Support: NIH grants AR055073, NS018956, and ES005022.


Nitrogen Mustard (NM; mechlorethamine hydrochloride) is a cytotoxic alkylating agent known to cause progressive pulmonary injury. Its toxic properties elicit extensive tissue remodeling within the respiratory tract, contributing to the development of chronic lung disease. Precision-Cut Lung Slices (PCLS) represent an ex vivo alternative to in vivo studies, minimizing animal numbers while increasing experimental replicates, which is advantageous for mechanistic toxicology studies and evaluating potential therapeutics. PCLS are three-dimensional en face sections of the lung that include all resident cell types; they remain viable in culture for up to 14 days, allowing for evaluation of functional activity, structural alterations and cytotoxicity. In the present studies, PCLS were used to assess NM-induced lung injury and alterations in pulmonary mechanics. C57BL/6 mice were euthanized, the lungs filled with agarose, isolated after congealment, and sliced (300 µM in thickness); slices were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) and Ham’s F-12 Nutrient Mixture (DMEM/F-12). Twenty-four hours later, PCLS were treated with NM (1, 10, and 100 µM) for one hour, washed, re-fed with medium and cultured for 3 days. NM caused a dose-related increase in cytotoxicity and a decrease in mitochondrial activity as measured by lactate dehydrogenase (LDH) and WST-1 assays, respectively. The decrease in mitochondrial activity was found to be dependent on the concentration of NM. Tumour necrosis factor α and granulocyte macrophage-stimulatory factor were increased in the medium and in the slices treated with NM. The expression of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) were also increased in the medium and in the slices treated with NM. The expression of VCAM-1 and ICAM-1 were also increased in the medium and in the slices treated with NM. However, the expression of VEGF and TGF-β was not affected by NM treatment. In conclusion, NM induced a dose-dependent increase in cytotoxicity and a decrease in mitochondrial activity in PCLS. These data demonstrate that PCLS can be used effectively to mechanistically assess the progression of injury induced by NM. Further studies, minimizing animal numbers while increasing experimental replicates, which is advantageous for mechanistic toxicology studies and evaluating potential therapeutics. PCLS are three-dimensional en face sections of the lung that include all resident cell types; they remain viable in culture for up to 14 days, allowing for evaluation of functional activity, structural alterations and cytotoxicity. In the present studies, PCLS were used to assess NM-induced lung injury and alterations in pulmonary mechanics. C57BL/6 mice were euthanized, the lungs filled with agarose, isolated after congealment, and sliced (300 µM in thickness); slices were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) and Ham’s F-12 Nutrient Mixture (DMEM/F-12). Twenty-four hours later, PCLS were treated with NM (1, 10, and 100 µM) for one hour, washed, re-fed with medium and cultured for 3 days. NM caused a dose-related increase in cytotoxicity and a decrease in mitochondrial activity as measured by lactate dehydrogenase (LDH) and WST-1 assays, respectively. The decrease in mitochondrial activity was found to be dependent on the concentration of NM. Tumour necrosis factor α and granulocyte macrophage-stimulatory factor were increased in the medium and in the slices treated with NM. The expression of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) were also increased in the medium and in the slices treated with NM. The expression of VCAM-1 and ICAM-1 were also increased in the medium and in the slices treated with NM. However, the expression of VEGF and TGF-β was not affected by NM treatment. In conclusion, NM induced a dose-dependent increase in cytotoxicity and a decrease in mitochondrial activity in PCLS. These data demonstrate that PCLS can be used effectively to mechanistically assess the progression of injury induced by NM. Further
understanding of the progression of damage and repair processes following exposure to mustard is essential in order to develop therapeutics for treating pulmonary toxicity. Supported by NIH AR055073 and ES005022.

### 2263 Investigating the Role of Mast Cell Activation by Nitrogen Mustard-Mediated Toxicity in the Lung

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Throughout war history, various chemical warfare agents (CWA) have been used leading to chronic effects on the immune system of veterans and civilians. One of the most notable CWA used in the Iran-Iraq war, Gulf War, and in recent conflicts in Syria is the vesicating agent, sulfur mustard (SM). Previously, studies demonstrated that mice exposed to SM exhibit an increase in pro-inflammatory cytokines followed by infiltration of neutrophils and macrophages in the lung. Dermal exposure to SM has reported to induce mast cell degranulation. As the first responding cells of the innate immune system, mast cells are derived from hematopoietic progenitor cell population of the bone marrow heavily populating the skin, eyes, and the lung. Therefore, the aim of this study was to determine if nitrogen mustard (NM): a surrogate for SM exposure promotes activation of mast cells causing chronic inflammation. Murine bone marrow derived mast cells (BMMCs) were used to assess early, intermediate and late phase activation through degranulation, eicosanoid expression, and pro-inflammatory cytokine production respectively. While NM exposure (1µM – 50µM) did not result in mast cell degranulation, we observed a 30-fold increase of cyclooxygenase-2 (COX-2) mRNA production at 6h following 10µM exposure NM. This resulted in downstream protein production of prostaglandin D2 (PGD2) as observed following 20µM-50µM exposure to NM at 6h. In addition, NM post 24h exposure to NM demonstrated a dose dependent increase from 10µM – 50µM. To further demonstrate an essential role for mast cells in NM exposure, we compared the effects of NM exposure on lung pathology between C57BL/6 and B6.Cg-Kitw-hsh/HiNihJaeB6J (mast cell deficient) mice. Significant lung injury was detected in the B6.NG/Ma and B6.Cg-Kitw-hsh/HiNihJaeB6J (mast cell deficient) mice. Significant lung injury was detected in NM treated B6.NG/Ma and B6.Cg-Kitw-hsh/HiNihJaeB6J (mast cell deficient) mice, whereas the B6.Cg-Kitw-hsh/HiNihJaeB6J (mast cell deficient) mice exhibited EHE staining. In contrast, significantly less injury was observed in mast cell deficient mice. These results suggest that mast cells play a prominent role in the injury induced by NM and may contribute to symptoms observed in veterans exposed to SM.

### 2264 Effects of Hydrogen Sulfide on Brain-Lung-Heart Axis

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Hydrogen sulfide (H2S) is a toxic gas with a rotten egg smell. Acute exposure to high concentration of H2S can lead to severe injuries including neurological disorders and acute death. The exact mechanisms underlying H2S-induced acute death have not been clearly elucidated. Hydrogen sulfide targets multiple organs including brain, lung, and heart. However, there is still a debate about the most critical organ responsible for acute death. In this study the effect of H2S on the brain-lung-heart axis was evaluated. C57BL/6J mice exposed to 1000 ppm of H2S by whole body inhalation developed clinical signs such as dyspnea, ataxia, and seizures. Pulmonary edema developed during H2S exposure and was evident up to 24 h post-exposure. Serum cardiac troponin-I was elevated at 2 h and 24 h post-H2S exposure, indicating cardiac injury. Blood samples were also collected for H&E stained sections. Phlebotomygraphy, electroencephalogram (EEG) and electrocardiography were simultaneously collected during H2S exposure to identify the sequence of the pathophysiology to identify the critical organ responsible to acute death. Respiratory distress and apnea were observed during exposure to H2S. Respiratory rate and minute ventilation decreased instantaneously, whereas the tidal volume decreased overtime. EEG results showed that brain activity was significantly and gradually suppressed over time during the H2S exposure. During seizure episodes, H2S exposed mice exhibited altered breathing pattern and arrhythmias. Interestingly, during knockdown mice exhibited significantly reduced EEG activity and apnea. Most significant finding was that the breathing pattern was first to be affected. Then brain activity ceased and closely followed by breathing cessation. Consistently, the heart continued to beat after brain activity and breathing had ceased in that order. Pre-treatment with midazolam prevented seizure and knock-down activity, allowing the H2S exposed mice to avoid sudden loss of consciousness and apnea. An unexpected finding was that midazolam pretreatment significantly reduced pulmonary edema in H2S-exposed mice. Taken together, these results indicate that brain death preceeds breathing cessation and cardiac failure. Therefore, the brain and lung are the most critical organs responsible H2S-induced acute death. Midazolam was shown to be beneficial both by reducing pulmonary edema and preventing seizure and knock-down activity. These results strongly suggest that countermeasures to increase survival should be directed at mitigating H2S-induced neurotoxicity and improving respiratory function.

### 2265 Repurposing Drugs as Countermeasures for Chemical Weapons: Interactive Training for Undergraduate Students

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The risk of a terrorist attack in the U.S. has created challenges on how to effectively treat toxicities that result from exposure to chemical weapons. To address this concern, the U.S. has organized a trans-agency initiative across academia, government, and industry to develop and approve drugs to treat tissue injury resulting from exposure to chemical threat agents. We sought to develop and evaluate an interactive educational session that provided hands-on instruction on how to re-purpose FDA approved drugs as therapeutics to treat toxicity from exposure to chemical weapons. Due to the COVID-19 pandemic, the Rutgers Summer Undergraduate Research Fellowship was run remotely for 6-weeks. In addition to independent virtual projects, students met twice weekly with instructors to participate in career development activities. Twenty undergraduate students participated in a two-hour session that included: 1) overview of the CounterACT program from the NIH Program Officer, 2) original research in novel methodologies to evaluate drug efficacy from a toxicology PhD student, and 3) an interactive session where teams of students were provided lists of FDA approved drugs to evaluate potential mechanisms of action and suitability as countermeasures for 4 chemical weapon case scenarios. The interactive session culminated in a competition for the best grant ‘sales pitch’. Pre- and post-program self-assessments using 5-point Likert rating scales were conducted online. Each participant had a unique identification number for the competition, and the judges were instructed to evaluate understanding of key programmatic objectives of the activity. From this interactive training, students improved their understanding of 1) the ability of chemical weapons to cause long-term toxicities (means: pre-1.5; post-3.6, p<0.0001), 2) how to apply the mechanism of action for a drug to a new clinical indication (means: pre-1.6; post-3.6, p<0.0001), and 3) re-purposing FDA-approved drugs to treat exposure to chemical weapons (means: pre-0.8; post-3.5, p<0.0001). Taken together, use of an interactive training exercise can provide students new insights into drug development for chemical weapon toxicities. Supported by R25ES020721, T22ES007148, P30ES050522, ULTR003017, U54AR055073 and the SOT and ASPET SURF Intern Programs.

### 2266 Chlorinated Tyrosine Adducts as Biomarkers of Chlorine Gas Exposure in a Swine Model

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Chlorine (Cl2) gas has been used as a chemical weapon since World War I and most recently in the Syrian conflict. In the United States, transportation and industrial accidents have caused multiple fatalities. Cl2 exposures cause severe injuries to the respiratory, cardiovascular, and nervous systems. However, biomarkers that specifically indicate Cl2 exposure remain to be identified and validated. Previous in vitro studies showed the formation of chlorinated tyrosine adducts such as 3-chlorotyrosine (Cl-Tyr) and 3,5-dichlorotyrosine (Cl2-Tyr) when human blood was exposed to Cl2. It is unknown whether these biomarkers are also formed in Cl2-exposed large animals such as swine, a species used as a translational model for human exposures, and whether these biomarkers are also formed in Cl2-exposed large animals such as swine, a species used as a translational model for human exposures, and whether these biomarkers are also formed in Cl2-exposed large animals such as swine, a species used as a translational model for human exposures. Due to the COVID-19 pandemic, the Rutgers Summer Undergraduate Research Fellowship was run remotely for 6-weeks. In addition to independent virtual projects, students met twice weekly with instructors to participate in career development activities. Twenty undergraduate students participated in a two-hour session that included: 1) overview of the CounterACT program from the NIH Program Officer, 2) original research in novel methodologies to evaluate drug efficacy from a toxicology PhD student, and 3) an interactive session where teams of students were provided lists of FDA approved drugs to evaluate potential mechanisms of action and suitability as countermeasures for 4 chemical weapon case scenarios. The interactive session culminated in a competition for the best grant ‘sales pitch’. Pre- and post-program self-assessments using 5-point Likert rating scales were conducted online. Each participant had a unique identification number for the competition, and the judges were instructed to evaluate understanding of key programmatic objectives of the activity. From this interactive training, students improved their understanding of 1) the ability of chemical weapons to cause long-term toxicities (means: pre-1.5; post-3.6, p<0.0001), 2) how to apply the mechanism of action for a drug to a new clinical indication (means: pre-1.6; post-3.6, p<0.0001), and 3) re-purposing FDA-approved drugs to treat exposure to chemical weapons (means: pre-0.8; post-3.5, p<0.0001). Taken together, use of an interactive training exercise can provide students new insights into drug development for chemical weapon toxicities. Supported by R25ES020721, T22ES007148, P30ES050522, ULTR003017, U54AR055073 and the SOT and ASPET SURF Intern Programs.
adducts were also significantly increased in the lungs of C1 exposed-pigs. These observations in swine models validate chlorinated protein adducts as biomarkers of C1 exposure, potentially serving as forensic biomarkers to validate human exposures.

2267 Organophosphate Pesticides as Potent CYP2C19 Inhibitors In Vitro

An estimated 300,000 people die of organophosphate pesticide (OPP) poisoning per year. Some of these OPPs are sold in their activated form, but often require Phase I metabolism by Cytochrome P450s (CYPs) to become activated. We have investigated the metabolism (intrinsic clearance) of 23 diverse organophosphate pesticides by CYP2C19. We compared our metabolism data with the inhibition of CYP2C19 by the same pesticides. We did not identify a clear correlation between metabolism and inhibition by CYP2C19 for these pesticides. However, we discovered for the first time that five of these OPPs are very potent inhibitors of CYP2C19 activity in vitro with IC50 < 20 nM. The most potent was phosalone (IC50 = 1.3 nM), which only displayed moderate metabolism. CYP substrate metabolism of a known substrate can be inhibited by other compounds they metabolize and or their metabolites in a competitive or in a more complex manner. This example of potent CYP2C19 inhibition by a molecule that is not efficiently metabolized suggests a role for mechanism-based inactivation, where a metabolite of phosalone is a more potent inhibitor of CYP2C19 than the parent molecule. This will be the focus of further evaluation to determine whether it is reversible or irreversible, alongside profiling of the OPPs with other CYPs in order to completely profile these compounds and understand the individual CYPs involved in metabolism and inhibition of each.

2268 Assessment of Corneal Alkali Injury in a Murine Model with Anterior Segment Optical Coherence Tomography (AS-OCT)
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Alkali chemical burns to the cornea can ultimately lead to blindness. Slit lamp exam is traditionally used to assess damage to guide management; however, it is limited by poor depth analysis and obstruction by tissue scarring. Anterior segment optical coherence tomography (AS-OCT) is a non-invasive, high-resolution imaging modality that can potentially be used to overcome these limitations and provide fast, depth-resolved evaluation of corneal wound healing and remodeling. Following an IACUC-approved protocol, acute injury model of alkali burn was performed on C57BL/6 mice (n=3) by applying a 2 mm diameter circle of filter paper soaked in 1.0M NaOH to the right eye, while the left eye was treated with PBS-soaked filter paper as a control. After 30 seconds, both eyes were flushed with saline. Pain management was performed with subcutaneous buprenorphine immediately after the procedure. AS-OCT was performed on the cornea and iris simultaneously, before and after the chemical burn, as well as at 7 and 14 days following the burn. Corneal thickness of each eye was measured at nine points on a central cornea slice in ImageJ and OCT angiography (OCTA) was performed on the images captured by AS-OCT using a custom algorithm. We determined that alkali burns result in increased corneal thickness immediately post-injury (+70.33%), peaking on day 7 (+105.02%) and regressing slightly on day 14 (+101.95%). In burned eyes, AS-OCT observed epithelial bullae and corneal opacity by day 7, and Descemet’s membrane detachment by day 14. In addition, beginning on day 7, OCTA showed development of neovascularization from the limbal area branching towards the center of the cornea. An intense anterior chamber inflammation was also noted, with hyper- and hyporeflective stromal cysts, as well as expanesive edemas, on day 7. AS-OCT was able to, with high sensitivity, detect edema/swelling, opacification, and neovascularization resulting from alkali burn. It also provided detailed cross-sectional information for non-invasively exploring angiogenesis and tissue remodeling, as well as 3D visualization of the anterior chamber and corneal layers, regardless of the amount of corneal scarring. AS-OCT can be a valuable adjunct to standard diagnostic tools for the assessment of corneal alkali injury and wound healing.

2270 Mechanism of Uptake and Cytotoxicity of Belantamab Mafodotin (Belamafl), an Anti-B Cell Maturation Antigen (BCMA) Antibody Drug Conjugate (ADC), in Primary Human Corneal Epithelial Cells
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Belamaf (BLENREP, formerly GS2287916) is an ADC comprising an anti-BCMA antibody conjugated to monomethyl auristatin F (MMAF), a potent microtubulin inhibitor, for the treatment of multiple myeloma. In clinical trials, ocular adverse events (AEs) were the most frequent AE, consistent with reports of other ADCs utilizing microtubulin cytotoxicity. We characterized underlying development of corneal AEs with ADCs containing MMAF and other microtubule inhibitor payloads are unknown. One hypothesis involves nonspecific ADC uptake into actively dividing cells of the corneal epithelium leading to apoptosis or inflammatory, which manifests as keratopathy and changes in vision. We investigated the mechanism of uptake and cytotoxicity of belamaf in a monolayer cell culture model of primary human corneal epithelial cells (HCECs). As a comparator, human primary renal proximal tubule epithelial cells (RPTECs) were used. GS2287914 (antibody minus MMAF payload) was used as a negative control for antibody cytotoxicity. Belamaf uptake and cytotoxicity was assessed by monitoring effects on apoptosis, cell viability and confocal microscopy in multiple donors of HCECs and RPTECs after 48 hours, with or without macropinocytosis inhibitor 5-(N-Ethyl-N-isopropyl)amiloride (EIPA). Uptake of belamaf in both cell types was concentration and time dependent and preceded significant concentration-dependent decreases in apoptosis. This is consistent with the mechanism via which MMAF payload is thought to induce cell death. Our results suggest belamaf-mediated cytotoxicity occurs via apoptosis following microtubulin inhibition in both RPTEC and HCECs in vitro. Given the lack of significant BCMA expression in these cells, a nonspecific belamaf uptake pathway is likely involved. The reduced uptake and amelioration of belamaf-mediated apoptosis with EIPA pre/co-treatment indicate that macropinocytosis plays a role in this nonspecific uptake. Funding provided by drug linker technology licensed from Seattle Genetics; monoclonal antibody produced using POTELLIGENT Technology licensed from BioWa. Abstract submission supported by Sarah Hauze, Fishawack Indicia Ltd, funded by GSK.

2271 A 1-Month Toxicology and Biodistribution NHP Pilot Study Evaluating a Single Subretinal Bilateral Administration of SPVN06—A Novel AAV-Based Gene Therapy for the Treatment of Rod-Cone Dystrophies Agnostic of the Causative Mutation
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Rod-cone dystrophies (RCD) are inherited neurodegenerative diseases characterized by an initial loss of rod photoreceptors (rods) followed by loss of cone photoreceptors (cones) eventually causing blindness. Greater than 1.5 million individuals worldwide are affected by RCD with 71 genes identified. By the time most patients visit the clinic, numerous rods have degenerated and many that are left lack function. The NXNL1 gene encodes 2 proteins, the rod-derived cone viability factor (RdCVF) and its full-length isoform, the thio-redoxin RdCVFL, produced by rods and rods/cones, respectively. These factors support cone survival by promoting glycolysis and preventing oxidative damage, respectively. SPVN06 is a novel AAV-based drug candidate encoding both human RdCVF and RdCVFL within the same vector. A single subretinal administration of SPVN06 is expected to protect against cone degeneration in RCD patients independent of the causative mutation. SPVN06 nonclinical safety was evaluated in a 1-month pilot toxicology and biodistribution study in non-human primates. Four female cynomolgus monkeys received a bilateral subretinal injection of vehicle (n=1) or SPVN06 (n=3) at 1 x 10¹¹ vg/eye (100 µL). The animals were observed for one month. A limited toxicological assessment was conducted to assess systemic and ocular safety. Parameters evaluated included clinical observations, body weight, hematology, clinical pathology, organ weights and macroscopic observations. Ocular, brain, and liver tissues were microscopically assessed. A comprehensive ocular evaluation including indirect ophthalmoscopy, slit lamp biomicroscopy, intraocular pressure (IOP) measurements, fundus imaging, optical coherence tomography (OCT) and full-field electroretinography (ffERG) was conducted. SPVN06 subretinal administration at 1 x 10¹¹ vg/eye (100 µL) was well tolerated. There were no
2272 Multifocal Electroretinographical Change of Laser-Induced Choroidal Neovascularization Model in Nonhuman Primates


Laser induced choroidal neovascularization (LCNV) model in non-human primates (NHPs) has been widely used to investigate wet age-related macular degeneration (wAMD) treatment in pharmacology field. FFA and OCT examinations are the main parameters to measure treatment effect. However, the visual functional change is hardly reported. LCNV only caused injury at the posterior pole of retina which might not affect full-filed ERG (fERG) much. Multifocal ERG (mERG) might be a more appropriate tool to evaluate functional change. To validate the mERG change in LCNV model in Cynomolgus monkeys treated with anti-VEGF substance. 6 Cynomolgus monkeys were assigned into two groups according to the FFA leakage. Argon laser of 532 nm was shot on the retina, 8 spots/eye. Anti-VEGF/Saline was delivered by intravitreal injection (ITV) 2 weeks post laser. Ocular Morphology: FDA, fundus photo (FP), and OCT. Entire Retinal Function: fERG with ISCEV standard protocol, Precise Retinal Function: mERG, Roland RetiMap. Four eyes in three animals with low rate of Grade 4 leakage (<2/8 or 25%) were excluded from the study on Day 15 (2 weeks post-laser). The grade 4 leakage rate was 53.1% in Control Group and 60% in Treatment Group. Fluorescin leakage were severe post Saline ITV throughout the study, while these were significantly relieved post Anti-VEGF ITV at retina loci where laser was shot. Compared to fERG, mERG is a more sensitive tool to assess laser affected retinal function change in the LCNV model in NHPs.

2273 Further Optimization of the OptiSafe Nonanimal Test Method

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OptiSafe™ is a shelf-stable, nonanimal test method to determine whether a chemical or mixture of chemicals is an ocular irritant. Previous studies have demonstrated that the method exhibits high sensitivity; however, the false-positive (FP) rate is around 40%, similar to other nonanimal ocular irritation test methods. The goal of the current study is to reduce the FP rate with specific formulation improvements. Based on a study of chemicals associated with FPs and their respective functional groups, a high percentage of OptiSafe FPs had aryl and ether functional groups and/or chemicals associated with an oxidizing chemical. Based on the high antioxidant capacity of the eye, we evaluated various antioxidants and determined that the FP rate could be reduced by including antioxidants in the formulation without impacting the false-negative (FN) rate. The updated version is now called GS2. Based on the in-house retesting of chemicals from a prior validation study, the specificity of the GHS not classified (NC) category improved from 60.0% (21/35) to 80.0% (28/35) using the new formulation, protocol, and prediction model. The sensitivity remained at 100% (38/38), and overall accuracy improved from 80.3% (57/71) to 90.4% (66/73). Based on the chemicals tested from the previous validation study and an expanded number of ocular corrosives for the detection of NC chemicals, the FP rate was 19.0% (8/42), the FN rate was 0.0% (0/81), and the accuracy was 93.5% (115/123). For the detection of ocular corrosives (GHS category 1), the FP rate was 26.9% (18/67), the FN rate was 9.1% (4/44), and the accuracy was 80.2% (89/111).

2274 Role of Aldo-Keto Reductases in the Nitroreduction of 1-Nitropyrene and 1,8-dinitropyrene


1-Nitropyrene (1-NP) and 1,8-dinitropyrene (1,8-DNP) are nitroarenes commonly found in diesel exhaust and classified as either probable or possible human carcinogens by IARC. Significantly, both compounds require metabolic activation by nitroreduction to generate metabolites capable of forming DNA adducts. Xanthine oxidase (XO) has been identified as one enzyme implicated in the nitroreduction of 1-NP and 1,8-DNP to form DNA adducts in rat liver; however, the enzymes involved in this process in human lung remain to be elucidated. We have previously shown that human aldo-keto reductases (AKR1C1, AKR1C2 and AKR1C3) and NAD(P)H quinone dehydrogenase 1 (NQO1) catalyze the nitroreduction of another diesel exhaust nitroarene, 3-nitrobenzanthrone (3-Nitro-7H-benzo[d]ethanthren-7-one, 3-NBA), raising the possibility that these enzymes could be involved in the nitroreduction of 1-NP and 1,8-DNP. Furthermore, studies have shown that AKR1C1, AKR1C2 and AKR1C3 are induced by the Nrf2-Keap1 pathway. In the current study, we showed that human recombinant AKR1C1 catalyzed 1-NP nitroreduction using a UV-HPLC-based discontinuous assay that measured the formation of 1-amino-1-nitro-o-cresol (1-AF), a product of 1-NP nitroreduction. By contrast, AKR1C2 and AKR1C3 catalyzed 1-NP nitroreduction to a more limited extent, while AKR1A1, AKR1B1, AKR1C4, AKR1C9, AKR1D1, AKR7A2, AKR7A3, and NQO1 had negligible nitroreductase activity with 1-NP as substrate. Investigation of 1,8-DNP nitroreduction showed that AKR1C3 was capable of this reaction; however, the reaction failed to go to completion within 24 hours. Other tested enzymes minimally catalyzed 1,8-DNP nitroreduction. Interestingly, our work showed that purified XO catalyzed 1-NP and 1,8-DNP nitroreduction poorly under either aerobic or anaerobic conditions. By contrast, XO catalyzed the robust reduction of cytochrome c. Ongoing work will further elaborate the role of nitroreductases in the metabolic activation of 1-NP and 1,8-DNP in human lung cells. Supported by R01-ES029294 (to TMP) and T32-ES019851 fellowship to ALS.

2275 Dose-Dependent Respiratory Impacts of Acrolein and TCE in Allergic Mice and Validation of In Vitro Effects


Animal models support validation of high-throughput in vitro assessments of hazardous air pollutants (HAPs) and other methodologically challenging chemicals. Acrolein (ACR) and trichloroethylene (TCE) are EPA high priority HAPs which may exacerbate respiratory symptoms in susceptible populations such as asthmatics. We evaluated real-time pulmonary responses to ACR and TCE in control and house dust mite (HDM)-allergic male (M) and female (F) Balb/cJ mice and subsequent inflammatory biomarkers using 2 protocols with different concentration x time (C x T) exposures. On 2 consecutive days, mice were exposed to nose-only inhalation air, followed by increasing concentrations of ACR (0.1, 0.32, 1.0, 3.2 ppm) or TCE (3.2, 10, 32, 100 ppm), corresponding to ongoing in vitro assessments. In protocol 1 (P1), exposures were 25 min at each concentration of ACR or TCE; in protocol 2 (P2) exposures were 7.5 min at the first 3 concentrations and 90 min at the highest concentration; total C x T was 2.6-fold higher in P2. Separate groups were exposed to air only to compare inflammatory responses. Baseline breathing frequency was higher in M vs. F mice; frequency significantly declined in all groups at 3.2 ppm ACR in either P1 or P2 (group means 37-67% lower than air baseline). ACR P1 reduced frequency to a greater extent in M mice, while reductions with P2 were equivalent in both sexes. TCE P1 and P2 (10-100 ppm) also significantly reduced frequency, though to a lesser extent (12-23%). Other parameters of respiratory timing, flows, and volumes indicated overall greater effects of ACR and TCE in M and allergic groups compared with F and control groups using either protocol. Four hours after final exposure, HDM-allergic F mice had greater induction of allergic inflammation than M mice (bronchial lavage eosinophils, lymphocytes, albumin, IL-5), but relatively few differences with respect to HAPs exposure, even with higher C x T in P2. Effects of ACR and TCE on real-time respiratory physiology correlated reasonably well with cell viability results in BEAS-2B cells (CellTiter Glo viability ATP assay). Assessment of additional HAPs in the allergic mouse model will further determine validity of HAPs effects predicted by high-throughput in vitro assays. This abstract does not represent US EPA policy.
Women and children of low socioeconomic status are disproportionately exposed to household air pollution. Fine particulate matter (PM$_{2.5}$) is a component of household air pollution with well-established health effects and is significantly elevated in households burning biomass fuel compared to non-biomass households. There is a lack of exposure-response data to determine the health impacts of different fuel sources. We performed a robust comprehensive characterization of particle size, composition, oxidative potential, and bioactivity assessments of PM$_{2.5}$ from biomass and non-biomass burning households in two communities in India. PM$_{2.5}$ was collected in 24 households using stationary monitors placed in kitchens and personal monitors worn by female and male participants, resulting in 6 groups stratified by fuel source and monitor type. Chemical analysis via quantification of polycyclic aromatic hydrocarbons (PAHs, n=115) and elements (n=75) was conducted and showed significant differences between fuel sources and monitor types. Elevated concentrations of oxy-PAHs were observed in PM$_{2.5}$ collected from biomass kitchen and female monitors compared to non-biomass counterparts. Assessment of oxidative potential was performed for the stratified groups as well as individual filters with significant differences observed between fuel sources and households. Bioactivity was determined using a range of concentrations in the developmental zebrafish assay (n=32/treatment) with mortality and morphological/behavioral changes being assessed at 24 and 120 hours post-exposure. An earlier incidence of significant mortality and morphological effects following exposure to PM$_{2.5}$ from biomass burning compared to the non-biomass households was observed. For all assessments, differences were observed between fuel type, highlighting the importance of selection of home heating and cooking sources. Data analysis is underway for zebrafish behavior. Associations between concentration, composition, oxidative potential, and bioactivity will be made and we anticipate specific constituents will be significantly associated with morphological and mortality endpoints. This research provides robust characterization of PM$_{2.5}$ that will be applicable to epidemiology studies to identify additional characteristics for health effects associations.
Wood smoke exposure causes respiratory distress including exacerbation of asthma, worsening of COPD, respiratory infections, and decreases in lung function. Currently, the number of individuals exposed to wood smoke is increasing, yet the cellular and molecular mechanisms remain poorly understood. We hypothesized that exposure to wood smoke condensate (WSC) would disrupt the barrier integrity of epithelial cells and engender an oxidative stress and inflammatory response in directly exposed epithelial cells and underlying lung fibroblasts. To test this hypothesis, we developed a Transwell-based in vitro organotypic model, combining both airway epithelial cells (16HBEs) and airway fibroblasts (IMR90 and primary human lung fibroblasts), which recapitulates both in vivo cellular architecture and intercellular signaling. We found that both epithelial cell layer polarization and high electrical resistance, low compound permeability, and polarized tight junction proteins before exposure to WSC; however, after 24 hours of exposure to WSC, epithelial cell electrical resistance is reduced without a change in small molecule permeability. We then investigated the effects of WSC exposure on the kinetics of gene and protein expression for oxidative-stress responsive and pro-inflammatory targets in airway epithelial cells and fibroblasts. WSC exposure induced several pro-inflammatory cytokines (IL-8, IL-6, IL-1α, and IL-1β) and redox-responsive genes (HMOX1, GCLM, SQSTM1, NQO1, and COX2) in both cell compartments. While the magnitude and kinetics of induction of pro-inflammatory and some transcriptional targets were similar in epithelial cells and fibroblasts, the expression of SQSTM1 was notably greater in epithelial cells and HMOX1 and COX2 were greater in fibroblasts. To discover the molecular events leading to changes in expression we then investigated the role of the redox-responsive NRF2, ERK, and P38 signaling pathways in epithelial cells and fibroblasts. Our data indicate that early (2 hrs) NRF2 response in both epithelial cells and fibroblasts, whereas WSC-induced ERK and p38 phosphorylation were sustained (2-24 hrs) in epithelial cells and peak early (2 hrs) in fibroblasts before returning to baseline levels. Together, these results indicate that WSC exposures have effects beyond directly exposed epithelial cells and that these two cellular compartments are affected differently in WSC exposures. Our findings provide novel insight into the roles of different cellular compartments in the response to inhaled toxics and suggest that lung fibroblasts should be considered as targets of inhaled chemical exposures.

I-81 is an elevated viaduct and goes through the heart of Syracusa with local businesses and communities located underneath. The safety limit of the via- duct was set, and the NY State DOT was looking for recommendations to rebuild the roadway using its current elevated design or lower the highway to ground level creating a community grid. People living below I-81 are concerned about increases in air pollution if I-81 is lowered. With no knowledge of current air quality levels in this community, assessing how lowering the highway would impact the neighborhood is impossible. We began an air quality monitoring campaign to measure pollutant levels simultaneously near the highway and on the ground below. Models generated by the DOT suggest traffic levels will not change if the highway is lowered. By monitoring air quality with elevation, results will offer a glimpse of potential air quality problems that could arise. Two different sampling campaigns were conducted to assess daily variation in traffic-related particulate matter (PM) near this highway at a building adjacent to I-81. The first campaign utilized two high-volume cascade impactors placed at ground and highway levels of a building to collect daily PM samples for two weeks over two seasons. Two real-time PM monitors measured PM number concentration with elevation. For fine PM at the rooftop location, the average number of particles was 1.2 times higher with a maximum of 10 times higher. Three days in summer had exceptionally high vehicle flow and had PM concentrations ranging from 4.5 to 10 times higher at highway level. Results from gravimetric analyses agreed with number concentration data. The study found that there was a pulley system to deploy up to six air quality and noise monitors at various heights along the building. This would allow us to determine how air quality and noise levels differ in a vertical fashion. Using mesh backpacks and a pulley system, we were able to conduct several trial runs and verify that we can monitor PM concentration and noise with elevation outside this building. In general, there were slight increases in particle mass concentration for particles less than one micron in diameter and 2.5 microns in diameter. For the noise levels, there were very few differences between the heights tested. Additional sam-
Epidemiological studies have reported the association of some volatile organic compounds (VOCs) exposure and cancer risks among residents living near petrochemical facilities. However, hundreds of VOCs are present in the ambient air at very low concentrations and may be released from a variety of sources. It is vital to develop a strategy to prioritize VOCs with relatively high risks and then to identify their emission sources. The Taiwan Environmental Protection Agency has established 17 monitoring stations to measure 109 VOCs around petrochemical industrial parks in Kaohsiung city of Taiwan since 2015. Based on VOC concentrations measured in these stations during 2015-2018, we calculated the annual mean concentrations of 109 VOCs. Then, we integrated probability risk assessment (PRA) and positive matrix factorization (PMF) model to identify the emission sources of VOCs with relative high cancer risks in these areas. First, we prioritized 10 out of 23 carcinogenic VOCs with the results of PRA. Utilizing the PMF model, we further found that petrochemical industrial parks contributed to more than 50% of emissions for 7 VOCs measured in some monitoring stations. These VOCs were 1,3-butadiene, benzene, naphthalene, 1,2-dichloroethane, chloroform, vinyl chloride, and acrylonitrile. Our present study demonstrated that analysis of tremendous data from monitoring stations with PRA followed by the PMF method allowed us to identify which VOCs emitted from the petrochemical facilities are of priority concern. This strategy will help regulatory agencies to efficiently control the emission of most concerned VOCs and to reduce the health risk for the residents near petrochemical industrial parks.

Fine particulate matter (PM$_{2.5}$) is a complex mixture of particles and sorbed chemicals that poses serious adverse health effects such as increasing cardiovascular and respiratory morbidity. There is ongoing research into the impacts of PM$_{2.5}$ of differing chemical compositions and the mechanisms for the observed health effects. To conduct these analytical and toxicology studies of PM$_{2.5}$, researchers split filters into sections. This process allows multiple, often destructive, assays to be performed. Our previous research showed chemical composition differences across a PM$_{2.5}$ filter, and therefore oxidative potential may have similar differences due to redox active metal concentrations. The goal of our study was to determine the validity of splitting filters for use in multiple analyses by assessing differences in chemical composition and oxidative potential within the same filter. Six PM$_{2.5}$ filter samples collected from urban and rural locations in Arkansas, United States were used. Each filter was split into quadrants, resulting in a total of 24 pieces, laboratory and blank filters were also prepared in the same manner. Each filter piece was extracted, concentrated, and a whole particle and soluble fraction were prepared. The extracted, whole particle suspensions and soluble fractions were then analyzed with dithiothreitol (DTT) assay run in triplicate to determine oxidative potential. Inductively coupled plasma mass spectrometry (ICP-MS) will be run on all samples and controls to compare chemical composition of the filter quadrants (n=30). Significant differences in oxidative potential between filter quadrants were not seen in the whole particle suspension and thus we do not expect to see significant differences in chemical composition. Oxidative potential analysis for the soluble fraction and elemental analysis are in progress. Correlation analysis between oxidative potential and elements will be conducted. This work will provide information about the feasibility of splitting PM$_{2.5}$ filters for multiple analyses on the same sample.

In 2015, the United States Environmental Protection Agency (USEPA) Maximum Achievable Control Technology (MACT) Rule mandated that all petroleum refiners monitor benzene levels along their fencelines. The main objective of this evaluation was to understand the seasonal variation in the benzene fenceline monitoring data collected in Corpus Christi, Texas. We utilized the benzene monitoring data from a refinery in Corpus Christi that reported to the USEPA for the years 2017-2020. Wind data were obtained from a nearby Texas Commission on Environmental Quality (TCEQ) air monitoring station (Corpus Christi Huisache), which is located southeast of the refinery. Analysis of the benzene concentrations from the 18 individual samplers around the refinery showed a seasonal variation, with the highest concentrations measured between the months of October and December. The average benzene concentrations for each quarter (January-March, April-June, July-September, October-December) indicate that quarter 4 consistently had the highest benzene concentrations for all the years analyzed. Based on the quarterly wind direction, the individual samplers were divided into up-wind and down-wind samplers. The winds were predominantly from southeast direction during quarters 1 to 3 (January - September), whereas winds were from both southeast and north directions during quarter 4 (October - December). The average benzene concentrations for the down-wind samplers were higher when compared to up-wind samplers across all quarters. The results indicate that the benzene concentrations at the passive samplers were higher during the winter season, likely due to weather conditions that occur more commonly in the winter, such as inversions. Benzene concentrations were also higher downwind of the refinery, demonstrating that the refinery contributed to the measured concentrations. With the trend in the average benzene concentration reducing over the years in Texas, better understanding of the seasonal and regional differences would enhance the identification of emission sources and protection of air quality and public health.
Epidemiological studies increasingly associate air pollution (AP) with neurodevelopmental disorders. Our previous studies indicate that developmental exposure to concentrated ambient ultrafine particles (CAPS) results in male-biased neurotoxicity, including enlarged lateral ventricles, altered corpus callosum myelination, and increased cell death. Constituents of CAPS leading to neurotoxicity are unknown, but particulate filter measurements revealed high levels of iron (Fe) and sulfur (S), both known to produce oxidative stress and be neurotoxic in excess in brain. To investigate the potential involvement of Fe and S in the developmental neurotoxicity of CAPS, mice were exposed to Fe2O3 nanoparticles and SO2 (Fe: 1.0 µg/m3, S: 0.66 mg/m3, based on human exposures) or CAPS (96 µg/m3) from postnatal days (PND) 0-10.13. Cell death in the nucleus accumbens (NAcc) at PND 14, 30, 60, and 90 was examined using caspase 3, a marker of apoptotic cell death, and FluoroJade, a non-specific cell death marker, due to the critical role of NAc in previously identified altered behaviors. At PND14, both CAPS and FeS exposure resulted in male-biased cell death, with a greater magnitude of effect for FeS than CAPS. Furthermore, FluoroJade and caspase 3 staining overlapped, indicating that cell death was likely apoptotic. However, future research should directly assess the potential for Fe triggered cell death through increased lipid peroxidation. Females had a decreased neuronal number at PND90, prompting examination of doublecortin, a marker of immature neurons, at PND 14. Females had significantly more doublecortin than males, and significantly decreased doublecortin following FeS exposure. These data support the hypothesis that FeS and CAPS alter cell death and maturation profiles in a sex-biased manner, which may underlie the sex-dependent alterations seen following developmental AP exposure. Understanding sex-dependent toxicity of AP is critical, as many children’s neurobehavioral disorders potentially linked to AP show sex biased prevalence rates. In total, evidence suggests that ultrafine particulates in AP may be contributing to neurodevelopmental disorders seen in humans, and further research is needed to identify the underlying cellular mechanisms of specific AP constituents, particularly metals.

Exposure via inhalation or dermal absorption of gunshot residue (GSR) has potential negative health effects in humans due to the organic and inorganic components found in GSR. Thus far there is limited information on size-differentiation in GSR particles which is a key factor in health effects research with particles less than 2.5 microns in aerodynamic diameter, PM0.3, being able to enter the respiratory system. This project collected size-selective PM0.3 and non-size-selective particles to determine the composition and presence of GSR as well as examine different methods of GSR collection. Air and hand swabs were collected during single and triplicate shots from a .22 caliber revolver to determine airborne and direct skin contact concentrations. To collect PM0.3, an ultrasonic personal air sampler (UPAS) with a 37 mm filter was used and double-sided tape was used to collect non-size selective particulates in the air. After the firearm discharge, hand swabs were collected by the shooter from gloves or by alcohol based handwipes. Following collection, the PM0.3 filter underwent black carbon analysis in triplicate using a Sootscan and the double-sided tape was used to characterize particle morphology and chemical composition with scanning electron microscopy paired with energy dispersive x-ray spectroscopy (SEM/EDS). All air and hand swab samples were then extracted in methanol via sonication for 60 min. Particle size distribution in the extracted solutions will be determined to establish differences in GSR between air and direct transfer to hands. Blanks for each collection media were used for all comparisons. The black carbon concentration on the PM0.3 filter collected during the 3 shots of the caliber revolver was 10 ± 40 ng/m3, while the single shot filter was below the detection limit. Non-size selective air sampling generated particles that were collected onto the double-sided tape after a single shot. The preliminary results suggest that multiple swabs provide adequate GSR to conduct several morphological and chemical analyses. Elemental analysis of all of the extracted samples are underway. The elemental results will give insight on the personal exposure to GSR as well as differences between direct contact and airborne concentrations. This research has identified collection methods and appropriate sampling procedures for future research into the impact that GSR has on human and environmental health.

The host response to inhaled entities is established through a delicate co-operation among professional immune cells and specialized epithelial cell layer of the respiratory tract that maintain homeostatic balance. Exposure to toxicants with immunomodulatory effects can disrupt mechanisms critical to host’s immune response and potentially impact susceptibility to lung diseases. Per- and polyfluoroalkyl substances (PFAS) are persistent chemicals that cause immunosuppression in mice and humans and have been associated with susceptibility to asthma and lung infections in humans. Emerging PFAS, including perfluoro-2-propoxy propanoic acid (GenX), are a concern for human health due to contamination of air and water. While both oral and inhalation exposures are acknowledged as routes of exposure, our understanding of PFAS-induced immune dysfunction is almost solely dependent on studies involving oral exposure. Because increasing evidence indicates that GenX is released into the atmosphere from industrial sources, it is critical that consequence of inhalation exposure to PFAS be investigated. In the current investigation, we hypothesized that GenX would impair the innate immune response to inhaled air pollution particles in mice. Male C57BL6 mice were exposed to saline vehicle, GenX (10 mg/kg), carbon black nanoparticles (CBNP) (4 mg/kg), or both GenX and CBNP via oropharyngeal aspiration. Lung tissue was harvested at 1- and 14-days post-exposure. Numbers of neutrophils in bronchoalveolar lavage fluid (BALF) induced by CBNP were reduced by GenX which coincided with reduced secretion of neutrophil chemokine CXCL1. Increased number of macrophages undergoing mitotic division was initially observed and was confirmed by immunohistochemistry analysis for Ki67, indicating that, indeed, the macrophages were undergoing proliferation. Data from the in vivo study with RAW264.7 murine macrophages correlated with the in vitro data, showing that GenX suppressed CXCL1 production yet stimulated cell proliferation. Overall, our study provides insight into GenX-induced suppression of the innate immune response of lung macrophages upon pulmonary exposure. Such outcome may impaire the host response to inhaled air pollution particles and promote lung disease susceptibility.

The inhalation of respirable uranium (U)-bearing particulate matter (PM) raises concern about the toxicological impacts and the health risks in communities affected by mining legacy. We synthesized respirable U-organic-bearing particulate matter (CBNP) via mixing uranyl nitrate (UO2(NO3)2) with GenX dispersate environmentally relevant to the mine-waste mineralogy at the Jackpile Mine, NM, and to understand the role of U and carbon (C) particulates in PM-induced toxicity. Human lung epithelial cells (A549) were exposed to different size range of synthesized U-organic-bearing PM (59% U and 22% C wt) as well as soluble U. The cells exposed to soluble U presented no cytotoxic effect while those exposed to U-organic-bearing PM showed significant cytotoxicity depending on U concentration (0-445 µg/mL). After 30 minutes of exposure, the wide size mixture (<0.2-10 µm) of U-organic-bearing PM showed 2.7 times higher U uptake than the narrow size mixture (0.2-0.85 µm) for cells exposed to 100 µM U. TEM-EDS analysis identified the intracellular translocation of large clusters of wide size U-organic-bearing PM (<0.2-0.9 µm) inducing significant DNA damage as indicated by pH2A-X fluorescence. These findings present new insights into the potential contribution of U-organic particulates into the genotoxicity and cytotoxicity of U, underlying the importance of understanding the environmental health impacts of atmospheric deposition in sites affected by U mining legacy.
Exposure to Traffic-Generated Air Pollution Increases Angiotensin II Pathway Signaling, Lipid Accumulation, and Inflammation in C57BL/6 Mice and 3T3-L1 Adipocytes, Which Is Exacerbated by Consumption of a High-Fat Diet

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Recent studies have reported a positive correlation between exposure to traffic-generated air pollution and metabolic syndrome and/or obesity in both children and adults; however, the signaling pathways involved are not yet well characterized. Systemic and local renin-angiotensin system (RAS) signaling is known to mediate an obese phenotype in adipocytes through altered 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 renin and Ang II, adipocyte hypertrophy, and adipocyte angiotensinogen (AGT) and AT-1 expression in C57BL/6 mice. Thus, we investigated whether MVE-exposure results in altered adipose lipid accumulation and induction inflammatory signaling pathways that contribute to the etiology of obesity. 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 PM/m3 diesel exhaust + 30µg PM/m3 gasoline exhaust (MVE n=10 per diet) for 6 hr/day for 30 d. Additionally, we applied plasma from these animals on 3T3-L1 mouse adipocyte cells in culture, ± pretreatment with an AT1 receptor antagonist, Losartan (10µg/mL), and assessed local RAS and lipid accumulation after 48 hrs. HF-diet significantly increased adipose lipid accumulation (~3-fold), as determined by Oil Red O staining, which was further exacerbated in MVE+HF diet animals (~4-fold), compared to FA animals. MVE-exposure also mediated significant elevations in adipose AGT, IL-6, and leptin, exacerbated by a HF diet, determined by immunofluorescence and RT-qPCR. When applied to adipocytes in culture, plasma from MVE+HF animals promoted adipocyte hypertrophy, lipid accumulation, AGT, and AT2 mRNA expression, compared to controls, which were normalized with Losartan pre-treatment. Our results suggest that exposure to traffic-generated pollution with a concurrent consumption of an HF diet high fat leads to alterations of adipocyte RAS signaling, adipocyte hypertrophy, and lipid accumulation associated with metabolic syndrome. Moreover, these alterations appear to be mediated (at least in part) through Ang II - AT1 signaling pathways. Funded by NIEHS R15ES026795 to AKL.

Exposure to Inhaled Diesel Exhaust Particles Alters Gut Microbiome and Intestinal Integrity, Which Is Mitigated by Probiotic Treatment in C57BL/6 Male Mice


Exposure to traffic-generated particulate pollution has been linked with gastrointestinal (GI) tract diseases; however, the pathways involved have not been fully characterized. Thus, we investigated the effects of inhaled diesel exhaust particles (DEP) on gut microbiome composition and intestinal barrier (IEB) integrity. Male 4-wk-old C57BL/6 wildtype mice fed a 45% fat diet were exposed via oropharyngeal aspiration to either 35µg DEP suspended in 0.9% saline or saline (SAL) control twice a week for four weeks. A subset of mice received 0.3g Ecologic® Barrier 849 probiotic (PRO) (7.5 x 10⁷ CFU/µL) or water (PLC) via drinking water daily. Microbial profiles were determined by 16S rRNA sequencing and compared to SAL controls, which were restored within PRO group. DEP-exposure resulted in a significant decrease in mRNA expression of ocludin, claudin-3, and zonula occludens (ZO)-1 in the duodenum, jejunum, and ileum. Circulating endotoxin levels were measured by chromogenic endotoxin kit. We observed an increase in Proteobacteria, Bacteroidetes, and Firmicutes. In contrast, there were no changes observed in occludin with DEP-exposure in PLC treated group. There was a significant increase in ocludin protein expression in DEP-exposed, compared to SAL controls within PLC group. In contrast, there were no changes observed in ocludin with DEP-exposure in PLC treated group. There was a significant increase in claudin-3 mRNA and protein expression in DEP-exposed mice compared to SAL controls within PLC but observed no change with DEP-exposure within PRO treated group. Interestingly, we noted differential expression of tight junction proteins throughout the portions of the small intestine, which were altered with PRO treatment. We also showed a significant increase in circulating endotoxin in DEP-exposed compared to SAL controls within PLC group, which was not observed within the PRO group. Our findings suggest that inhaled DEP results in altered gut microbiota and IEB integrity, which can be mitigated using probiotics. Funded by NIEHS R15ES026795 to AKL.

Exposure to Traffic-Generated Pollutants Exacerbates the Expression of Factors Associated with the Pathophysiology of Alzheimer’s Disease in Aged C57BL/6 Wild-Type Mice


Studies report a correlation between traffic-generated air pollution and detrimental outcomes in the central nervous system (CNS), including Alzheimer’s disease (AD). AD pathology in the brain is characterized by accumulation of amyloid-β (Aβ) plaques, oxidative stress, neurodegeneration, and neuroinflammation. Aβ is produced in the brain via the proteolytic cleavage of amyloid-β protein precursor (AβPP) by β-secretase enzyme (BACE1). Excessive renin-angiotensin (Ang) II signaling, through the Ang II type 1 (AT1) receptor, is associated with oxidative stress, cognitive impairment, and increased Aβ production in the CNS, all of which are hallmarks of AD. Furthermore, increased aryl hydrocarbon receptor (AhR) expression and activation have also been associated with inflammatory signaling in the CNS, aging, and AD. We have previously reported that exposure to a mixture of gasoline and diesel engine emissions (MVE) promotes blood-brain barrier disruption, increased cerebral microvascular Ang II - AT1 receptor signaling, and neuroinflammation in C57BL/6 mice. Therefore, we investigated the hypothesis that MVE-exposure exacerbates factors involved in the progression of AD pathology in the aged CNS. To test this, young (2 mo.) and aged (18 mo.) male C57BL/6 mice were exposed to either MVE (300 µg PM/m3) or filtered air (FA) for 6 h/day, 7 d/wk, for 50 d. Immunofluorescence and RT-qPCR were used to quantify AβPP, oxidative stress (8-OHdG), AT1 receptor, AhR, Aβ, and BACE1. Our results show increased cerebral AβPP mRNA and oxidative stress in the hippocampus of aged C57BL/6 mice, which was further exacerbated by MVE-exposure, compared to the CNS of young mice. Furthermore, MVE-exposure resulted in significantly increased AT1, associated with elevated AhR and BACE1 expression, and Aβ accumulation in the CA1 region of the hippocampus of aged C57BL/6 male mice, compared to the aged FA controls and young groups. Collectively, these findings suggest that inhalation exposure to traffic-generated air pollution exacerbates the expression of factors associated with progression of AD in the aged hippocampus, which is associated with increased expression of members of the RAS signaling pathway. Further mechanistic studies are underway to identify regulatory pathways altered by traffic-generated air pollution that may contribute to AD pathogenesis. Funded by NIEHS R00ES016586 and R15ES026795 to AKL.

Toxicity Induced by 9,10-phenanthrenequinone in Human Lung Epithelial Calu-1 Cells via Mitochondrial Dysfunction and Oxidative/ER Stress Pathways

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9,10-Phenanthrenequinone (9,10-PQ), a redox-active polycyclic aromatic hydrocarbon found in diesel exhaust particles and cigarette smoke, is known to play an important role in the adverse health effects triggered by air pollution. The toxicity of 9,10-PQ is associated with the generation of reactive oxygen species (ROS) via the process of redox cycling by enzymes such as cytochrome P450 and reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. 9,10-PQ is a potent inducer of lipid peroxidation, a major cellular event in cellular injury. Following this event, the one-electron reduction of 9,10-PQ by these enzymes generates a semiquinone; this species (ROS) via the process of redox cycling by enzymes such as cytochrome P450 and NADPH oxidase. This process generates a semiquinone radical. This radical then donates an electron to diatomic oxygen forming superoxide anion. This in turn generates cytotoxic oxidizing species including hydrogen peroxide, hydroxyl radicals and, in the presence of nitric oxide, peroxynitrite. In the present studies, we investigated mechanisms of 9,10-PQ toxicity in human lung epithelial Calu-1 cells. 9,10-PQ treatment caused a time- and concentration-dependent decrease in cell viability (IC50 = 25 µM after 4 h and 2 µM after 24 h). This was associated with a decrease in intracellular glutathione (GSH) and an increase in the ratio of GSSG to GSH. 9,10-PQ also caused a concentration-dependent decrease in intracellular ROS and lipid peroxidation products, as measured by CM-H2DCFDA and BODIPY fluorescence assays, respectively. Western blotting revealed that 9,10-PQ triggered endoplasmic reticulum (ER) stress and activation of mitogen-activated protein kinase (MAPK) signaling in Calu-1 cells, which was identified by enhanced

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phosphorylation of several key mediators including eIF2α, p38, ERK1/2 and JNK. In addition, 9,10-PQ induced mitochondrial dysfunction in the cells causing a reduction of mitochondrial membrane potential, depletion of ATP, and inhibition of oxygen consumption and glycolytic metabolism. N-acetyl cysteine pretreatment partially protected Calu-1 cells from 9,10-PQ toxicity by decreasing levels of ROS production, preventing mitochondrial dysfunction, and suppressing the activation of oxidative and ER stress pathways. Taken together, these data indicate that 9,10-PQ induces cytotoxicity by a mechanism involving mitochondrial dysfunction and ROS-mediated ER stress and MAPK signaling. Support: NIH grants AR055073, NS108956, ES040738, and ES005022.

2296 Prenatal Exposure to Ultrafine Particulate Matter Causes Sustained Growth Suppression in Offspring

Exposure to particulate matter (PM) air pollution during gestation is associated with increased risks for offspring developmental effects, including low birth weight and alternatively in some cases, metabolic dysfunction later in life (i.e., obesity and insulin resistance). To investigate these outcomes and potential underlying mechanisms, we carried out a pilot study in our established in utero inhalation exposure model. C57BL/6 female mice were timed-mated and randomly assigned to either filtered air (FA) or aerolized ultrafine PM (500 µg/m3) for 6 h/day from gestation day (GD) 0-18. PM was composed of diesel soot/black carbon, sulfates, nitrates, and chlorides to represent real-world components of traffic-related air pollution. After parturition, offspring were culled to 2 males and 2 females per litter on post-natal day 5 (PND). Offspring were next weaned on PND21, and subsequently assigned to either a high or low-fat (HF) diet (16 wk). We measured monthly glucose tolerance and insulin resistance tests to assess for changes in glucose homeostasis. Weight gain and feed intake was tracked weekly. Upon sacrifice at 15 weeks of age, organs were weighed and collected for future analyses. Mouse growth trajectories differed in respect to sex, diet, and prenatal exposure. Male mice displayed higher growth trajectories in comparison to FA-exposed males in both diet groups. This pattern was also observed within female mice and was more pronounced. These data support a growth suppression effect of in utero PM exposure that applies to both males and females that is influenced by diet. Tissues collected from PM-exposed mice revealed significant differences in relative weights of organs, including spleen, brain, brown adipose tissue (BAT), and white adipose tissue (WAT). Further analysis indicates differences in relative tissue weights based on high-fat and low-fat diets, as expected. In our model, we did not detect significant differences in monthly glucose tolerance or insulin resistance tests. As demonstrated in other models, we did not observe a propensity for obesity, which could be due to our PM composition, concentration or dosing window. Research is ongoing to evaluate body and bone composition longitudinally in our model.

2297 Omega-3 Fatty Acids Modify the Ambient PM<sub>2.5</sub> and Ozone-Induced Inflammation and Fibrinolysis in Young Healthy Adults
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Air pollution exposure has been associated with adverse cardiopulmonary effects. Omega-3 fatty acid supplementation has been shown to blunt cardiopulmonary responses to air pollution exposure. We conducted a panel study to evaluate whether personal choice of omega-3 fatty acids intake could modulate the health effects of short-term exposure to ambient air pollution in young healthy adults. Sixty-two healthy adults (mean age 38±9 yr) were divided into high (n=34) or low (n=28) omega groups based on their blood omega-3 index. Systemic inflammation and fibrinolysis were measured using a multi-effect approach and included a 5-day low- and high-dose intervention. Levels of IL-6 (P<0.01), TNFα (P<0.05), and fibrinogen (P=0.02) were dose-dependently increased in the high omega group (e.g., for an interquartile range (IQR) increase in IL-6, IL-6 was increased in the high omega-3 group. We observed differential effects of PM<sub>2.5</sub> and O<sub>3</sub> on inflammatory and fibrinolysis markers which was modulated by levels of omega-3 fatty acids. The effects were found at concentrations well below the National Ambient Air Quality Standards for both PM<sub>2.5</sub> and O<sub>3</sub>. Omega-3 fatty acids may offer protection against adverse health effects of ambient air pollution in young healthy adults. This abstract does not necessarily reflect US EPA policy.

2298 Oxidative Stress or Inflammatory Reaction in a Human Colorectal Cell Line or a Human Keratinocyte Cell Line Treated In Vitro with 5- or 50 μm Diameter Polyelectrolyte
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Microplastics (MPS) have been recognized as a global environmental threat and its exposure as a risk factor to human health. Health effects through MPS exposure have been recently reported, especially through oral route of exposure. Since MPS could be exposed to humans through routes other than oral, this study was designed to evaluate whether MPS exposed through various routes could induce oxidative stress or inflammatory reaction in cells at port of MPS entry. The cells lines used were Caco-2 human colorectal adenocarcinoma cell line, A549 human adenocarcinomic alveolar basal epithelial cell line, and HaCaT human keratinocyte cell line. The Caco-2 and A549 cells were activated with lipopolysaccharide (1 µg/mL/1.5 x 10<sup>5</sup> cells) for 24 cells following 24 stabilization period, and HaCaT cells were proceeded simultaneously. Polyelectrolyte (PE) with 5 µm or 50 µm diameter was added just before initiating stimulation at concentrations of 0.01x, 0.1x, 0.5x, or 1x lethal concentration causing cell death (LC50). In 25% (LC25), these concentrations were determined through CCK-8 assay. The LC25s were 1000 µg/ml for all the cell lines and both sizes of MP. Levels of intracellular reactive oxygen species were dose-dependently upregulated only in the Caco-2 cells treated with 5 µm diameter PE (0.01x: 9%±5%, 0.1x: 12±8%, 0.5x: 12±9%, 1x: 14±2% vs vehicle control), and no differences were found for the Caco-2 cells treated with 50 µm PE and the other cell lines. Concerning production of IL-6, a representative proinflammatory cytokine, no significant alteration was observed with A549 cells treated with 5 and 50 µm diameter PE, and no IL-6 production was observed with Caco-2 cell. On the other hand, HaCaT cell demonstrated a dose-dependent upregulation of IL-6 production following treatment of 5µm PE (vehicle: 43±6, 0.01x: 85±12, 0.1x: 97±20, 0.5x: 100±27, 1x: 90±11 pg/ml) and 50 µm PE (vehicle: 44±8, 0.01x: 90±10, 0.1x: 94±11, 0.5x: 102±12, 1x: 95±3 pg/ml). Overall, the present study suggests that MPS could induce oxidative stress or inflammatory reactions depending on the route of entry into human body. Supported by Korea Environmental Industry & Technology Institute Project No. RF20200413.

2299 Exposure to Concentrated Ambient Particles Promotes Telomere Length Shortening in Multiple Murine Cell Types
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Exposure to fine airborne particulate matter (PM<sub>2.5</sub>) is associated with a wide range of cardiovascular, neurological, and immunological impairments. At the cellular level, these adverse physiological outcomes may be a consequence of PM<sub>2.5</sub>-induced senescence and dysfunction. To determine if PM<sub>2.5</sub> exposure promotes cellular aging and senescence, we used a PCR method to monitor telomere lengths in cells isolated from mice exposed to filtered air or concentrated ambient particles (CAPs). We found that relative telomere lengths were reduced in peripheral blood mononuclear cells (PBMC) isolated from mice exposed to CAPs compared with those in the air-exposed controls. Reduced telomere lengths were also observed in two progenitor cell types, bone marrow c-kit+ cells and bone marrow endothelial progenitor cells, isolated from the CAPs-exposed mice. Furthermore, the reduction of telomere length in EPCs was particularly due to reduced telomerase reverse transcriptase activity. We have previously shown that the histidyl depeptide, carnosine, which protects from the deleterious effects of oxidative stress, mitigated CAPs-induced EPC functional impairments in mice. In the current study, we found that carnosine supplementation also limited CAPs-induced telomere length reduction in PBMC, c-kit+ cells, and bone marrow EPCs. These results suggest that PM<sub>2.5</sub> exposure can promote telomere length shortening in multiple cell types and that a practical intervention to limit the consequences of oxidative stress can mitigate these outcomes.
People are exposed to numerous forms of particulate matter (PM) in the course of daily activities. Exposures can contain coal fly ash (CFA), diesel exhaust particles (DEP), cigarette smoke PM (CSPM), calcium oxide (CaO), wood smoke PM (WSPM), and much more. In the lung, activation of transient receptor potential (TRP) channels are one mechanism by which PM can cause adverse effects. Human TRPV3 is activated by the soluble agonist drofeline as well as WSPM. TRPV1 is activated by the soluble agonist nonivamide and CFA. Human TRPA1 is also activated by CFA, DEP, and WSPM, as well as the soluble agonist allyl isothiocyanate (AITC). We have observed that responses of TRP channels to PM differs across species, particularly rodents and humans. This variability could have implications when attempting to translate toxicological findings from mice to humans, and vice versa. Alignment of human and mouse TRPA1, V1 and V3 amino acid sequences illustrated differences in selected amino acids in the pore-loop regions of these TRP channels, and it was hypothesized that these differences may contribute to differences in the response to PM. Mouse and human TRPA1, V1 and V3 plasmids, and mutants thereof, were transiently transfected into GCAM-HEK-293 reporter cells and calcium flux assays were performed to examine the base for species-specific differences in response of these TRPs to PM and known soluble agonists. For mouse TrpV3 the response to WSPM was decreased compared to human, and this decrease involved amino acids 612 and 616 of the pore-loop region. Mouse TrpV1 also had reduced responses to CFA and CaO compared to human TRPV1. The amino acid residues 605, 609, and 619, also in the pore-loop region, were involved, while residues 609 and 619 dictated the response to CaO. Interestingly, mouse TrpA1 exhibited increased responses to DEP, CSPM, WSPM, and CFA when compared to human TRPA1. Mutation of amino acid residue 939 in human TRPA1, to the corresponding mouse residue, reproduced the increased response to CFA. Identification of specific PM-sensing sites on TRP channels furthers our knowledge of the mechanism by which these channels are activated by PM and helps us understand potential limitations of animal models in assessing how TRP channels might contribute to human lung damage and disease. Further identification of sites forms us of ways to potentially modify rodent TRPs, via transgenics, to better reproduce the effects PM has on TRP activity and associated toxicological effects. Support: ES017431, ES027015.
2304 Systematic Review of the Association between Long-Term Exposure to Ambient Fine Particulate Matter and Mortality

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Over the last several decades, the epidemiology literature has evaluated associations between ambient fine particulate matter (PM2.5) and mortality. We recently developed a transparent systematic review and causality framework that incorporates best practices for evaluating study quality, evaluating and integrating evidence, and making causal determinations. We used this framework to conduct a systematic review of epidemiology studies of long-term exposure to ambient PM2.5 and mortality. We identified 485 studies and controlled for non-accidental exposure. We evaluated the quality of each study using a predetermined set of study quality criteria, to allow for a consistent evaluation across studies. We assessed the results of the studies in the context of their methodological strengths and limitations and evaluated the reliability of each study’s results for informing potential causality. We then integrated the evidence across studies using modified Bradford Hill aspects as a framework to systematically evaluate the weight of the evidence. Finally, we used a four-tiered causality framework to assess whether there is a causal relationship between long-term exposure to ambient PM2.5 and mortality. The results of this analysis indicate that there is some consistency across studies reporting positive associations, but these associations are weak. In addition, several important methodological issues in these studies, including potential confounding by measured and unmeasured factors, exposure measurement error, and model misspecification, render the evidence they present uncertain. Because these uncertainties render a plausible, alternative explanation for the weakly positive findings across studies, other than causality, we concluded that the evidence from these studies for a causal relationship between long-term exposure to ambient PM2.5 and mortality is inadequate. Our analysis indicates that a relatively consistent pattern of weak, positive associations does not necessarily lead to a conclusion of causality when study quality is incorporated into the evaluation, evidence from multiple studies is integrated in a consistent manner, and alternative explanations for the evidence are explored.

2305 Regulation of Pulmonary Surfactant and Lipid Homeostasis by PPARY in a Mouse Model of Ozone-Induced Lung Injury


Ozone is a ubiquitous urban air pollutant that causes airway inflammation and hyperresponsiveness. Evidence indicates that alterations in lung lipid homeostasis due to dysregulated catabolism by alveolar macrophages and/or disrupted recycling by alveolar epithelial type II cells drives inflammation and chronic lung injury. RNA-seq analysis of alveolar macrophages revealed that exposure of mice to ozone resulted in alterations in inflammatory and lipid metabolism pathways, and down-regulation of PPARY, a nuclear transcription factor important in controlling lipid metabolism, macrophage activation, and inflammation resolution. In the present studies, we analyzed the role of PPARY in ozone-induced alterations in lung lipid homeostasis. Female C57BL/6J mice were treated with the PPARY agonist, rosiglitazone or vehicle control by daily intraperitoneal injection beginning 24 hr prior to exposure to ozone (0.8 ppm, 3 hr) or air; mice were euthanized 72 hr post ozone. Bronchoalveolar lavage fluid (BAL) was collected and analyzed for total protein and phospholipid content, and expression of surfactant proteins. Effects on pulmonary function were assessed using a SciRes Flxivent. Ozone exposure resulted in increases in total protein and phospholipid content in BAL, consistent with lung injury and dysregulation of lipid metabolism. An increase in levels of the hydrophilic collectin, surfactant protein D (SP-D) and a decrease in the hydrophobic surfactant protein B (SP-B) were also observed in BAL after ozone exposure. SP-B and phospholipids regulate pulmonary function by maintaining lung surface tension and hysteresivity. Ozone exposure caused a significant reduction in hysteresivity at positive end-expiratory pressures of 1 and 3 cm H2O, indicating reduced viscoelasticity of the lungs. Rosiglitazone administration significantly reduced BAL phospholipid content and restored SP-B to control levels. Consistent with these findings, rosiglitazone rescued ozone-induced impairment of lung function. Collectively, these results suggest that ozone exposure disrupts SP-B and phospholipid homeostasis which impair pulmonary function; PPARY improves lung function by restoring surfactant and lipid balance. Supported by NIH ES004738, ES005022, ES029254, ES007148, HL086621, and ES030984.

2306 Social Isolation but Not Mild Chronic Stress during Early Life Exacerbate Ozone-Induced Metabolic and Immunological Effects in Rats

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Epidemiological studies have shown that psychosocially stressed individuals have exacerbated responses to air pollution exposure. Our lab and others have recently demonstrated that ozone exposure activates neuroendocrine stress response pathways leading to systemic metabolic and inflammatory responses in rats and humans. The purpose of this study was to identify whether community level stressors (i.e. noise, uncomfortable living conditions, confinement, fear) can interact with ozone to increase susceptibility for immunological and metabolic effects. Male, 4-week-old Wistar-Kyoto rats were randomly assigned into 3 stress groups: No Stress (NS) - control group; Social Isolation (SI) - single-housed; Chronic Stress (CS) - single-housed and subjected to mild unpredicted-randomized stressors (i.e. restraint, tilted cage, shaking, intermittent noise, and predator odor) 3 days/week for 8 weeks. Animals were then exposed acutely to filtered air or ozone (0.8 ppm) as a challenge stressor for 4h followed by necropsy. After 3 weeks of the stress protocol through the end of the study, the CS group had significantly decreased body weight compared to the NS and SI groups. This corresponded with increased adrenal weight and urinate corticosterone and metabolite levels measured in the CS group, suggesting the chronic mild stress protocol was effective. SI and CS tended to deplete circulating thyroid stimulating hormone, luteinizing hormone, prolactin, follicle stimulating hormone, and brain-derived natriuretic factor, but not adrenocorticotropic hormone in air-exposed rats (SI>CS). SI also induced more severe systemic inflammation than CS, as evidenced by increases in circulating cytokine levels (i.e. IL-1β, IL-4, IL-5, IL-6, IL-10, IL-13, and INF-γ) regardless of exposure. Ozone exposure led to increases in plasma corticosterone levels (NS and SI), plasma epinephrine (CS), and urine corticosterone and catecholamine metabolites (all groups). Exposure to ozone also led to increases in serum glucose, leptin, and branched-chain amino acids, increases in BALF IL-6 and TNF-α; and decreases in serum thyroid stimulating hormone, prolactin, and luteinizing hormone, with small influence of CS and SI. Systemic inflammation and some pulmonary effects of ozone were exacerbated by SI. Collectively, these data suggest that psychosocial stressors affect the neuroendocrine system and will likely sway the adverse metabolic and inflammatory responses induced by ozone exposure, a challenge stressor. Does not reflect the US EPA policy.

2307 Neuroendocrine Aspect of Ozone Adaptation at Lung and Systemic Level: The Influence of One-Month Glucocorticoid Pretreatment

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Ozone-induced lung injury and inflammation dissipate despite continuation of exposure for 3 or more days; however, the mechanisms of adaptation/habituation remain unclear. We have shown that ozone-induced pulmonary and systemic effects are mediated through reversible neuroendocrine activation and adrenal release of the stress hormones corticosterone and epinephrine. Here, we hypothesized that adaptation from ozone-induced pulmonary and systemic effects is associated with diminution of neuroendocrine activation and reduced glucocorticoid activity. Male Wistar Kyoto rats (12-week-old) were divided into 4 stress groups: No Stress (NS); Social Isolation (SI); Single-Housed (SH); and Chronic Stress (CS). After 3 weeks of the stress protocol through the end of the study, the CS group had significantly decreased body weight compared to the NS and SI groups. After 3 weeks of the stress protocol through the end of the study, the CS group had significantly decreased body weight compared to the NS and SI groups. SI and CS rats were then exposed acutely to filtered air or ozone (0.8 ppm, 3 hr). Collectively, these data suggest that psychosocial stressors affect the neuroendocrine system and will likely sway the adverse metabolic and inflammatory responses induced by ozone exposure, a challenge stressor. Does not reflect the US EPA policy.
Ozone (O₃) is a prevalent air pollutant that has been associated with increased incidence and exacerbations of cardiopulmonary diseases. O₃ exposure is known to promote pulmonary inflammation and injury. Following inflammation, resolution responses are activated to return the injured lung to homeostasis and prevent chronic inflammation and disease. Specialized pro-resolving mediators (SPMs) are a class of fatty acid derivatives, which promote tissue resolution and homeostasis. SPMs promote resolution by binding to G-protein receptors including ALX/FPR2, BLT-1 and ChemR23 to facilitate the downregulation of pro-inflammatory responses and upregulation of anti-inflammatory/pro-resolving mediators.

Recently, we have shown that O₃ exposure downregulates the pulmonary expression of the SPM receptor ChemR23. ChemR23 binds Resolvin E (RvE) SPMs as well as the chemokine Chemerin which is secreted by adipocytes and acts as a chemoattractant directing immune cells towards the site of inflammation. Interestingly, compared to WT mice, ChemR23-/- mice had decreased BAL protein levels indicating less pulmonary injury following O₃ exposure. Our results indicate that decreased expression of ChemR23 in O₃-exposed mice had significantly more chemerin in the airspace when compared to WT controls.

No differences were observed between WT and ChemR23-/- mice in markers of pulmonary inflammation. Interestingly, compared to WT mice, ChemR23-/- mice had decreased BAL protein levels indicating less pulmonary injury following O₃ exposure. Our results indicate that decreased expression of ChemR23 in O₃-exposed mice had significantly more chemerin in the airspace when compared to WT controls.

Future studies will investigate the underlying mechanisms by which chemerin/ChemR23 regulate O₃-exposed lung injury.

**2309 Phenotypic Analysis of Macrophages in Human Sputum after Ozone Exposure**


Following ozone exposure in rodents, a persistent increase in proinflammatory macrophages is observed in the lung. Moreover, inhibiting the activity of these cells mitigates toxicity, demonstrating that these cells play a key role in the pathogenic response to ozone. The role of inflammatory macrophages in ozone toxicity in humans is unknown. To begin to address this, in the present studies, we analyzed the phenotype of lung macrophages in induced sputum from healthy humans following exposure to air or ozone in a controlled environmental facility. Subjects (n=27) were exposed for 3 hr to filtered air or ozone (0.2 ppm) two weeks apart in a randomized crossover design. During exposures, subjects performed intermittent mild to moderate exercise. Sputum was collected from subjects 24, 48 or 72 hr after exposure. Mucus plugs were manually separated from sputum, weighed, and incubated in dithiotreitol (0.1%). Recovered cells were processed for histological analysis and flow cytometry. CD45+ viable cells were classified into 3 discrete populations: resident macrophages, recruited macrophages, and neutrophils, on the basis of CD14/CD16 positivity. Ozone exposure resulted in increased numbers of recruited macrophages present in sputum collected at 24 and 48 hr post-exposure. Phenotypically, recruited macrophages exhibited significant (*) p=0.05) increased CD206 expression in response to ozone at 24 hr (32.7±9.38 % 0, 48.63±9.14 % 0 and 72 (27±6.11± 6.0 % 0, post-exposure compared to air, suggesting phagocytic activity. In resident macrophages, CD11b expression was significantly increased 24 hr post-ozone (45.5±15.0 % 0, as indicated by the % difference between air and ozone exposure for each subject; at later time points, CD11b expression trended towards baseline (23.6±14.0, 10.4±10.2). Phenotypic changes in resident macrophages and an influx of recruited macrophages to the lung with their subsequent activation, as indicated by increased CD11b expression, reflects an immunomodulation in humans in response to ozone injury. Functional analysis of purified cell populations will facilitate a greater understanding of the role of macrophages in promoting lung injury and inflammation after ozone exposure. Supported by NIH ES004738 and ES050522.

**2310 Fine Particulate Matter Exposure-induced Adipose Tissue Changes in Diet-induced Obesity and Light-induced Circadian Dysynchrony**

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Exposure to fine particulate matter (PM₂.₅) air pollution is associated with an increased type-2-diabetes (T2D) risk. Both, having bad sleeping habits and choosing an unhealthy diet are significant factors that contribute to the world-wide increase in T2D. Although current research indicates that air pollution exposure exacerbates metabolic injury induced by lifestyle choices such as bad sleep and dietary habits, it is still unclear how PM₂.₅ exposure increases the susceptibility to develop insulin resistance and diabetes. Hence, we examined whether and how PM₂.₅ exposure increases glucose intolerance and insulin resistance in light-induced circadian dysrhythmia (LID) or diet-induced obesity (DIO). Control mice (12/12h, light/dark; 12% kcal from fat, control diet) and mice maintained either on a disturbed light cycle (18/6h, light/dark; LD) or on a high fat diet (60% kcal from fat, DIO) were exposed to concentrated ambient PM₂.₅ (CAP, 30 days, 6h/d) or HEPA-filtered air and systemic and organ specific changes were examined. As indicated by a glucose tolerance test or calculated HOMA-IR scores, exposure to CAP exacerbated systemic glucose intolerance and insulin resistance in both LID and DIO mice but not in non-obese mice kept on a regular light cycle. These results confirm that CAP exposure exacerbates systemic insulin resistance in LID and DIO. Interestingly, while CAP exposure decreased adiposity and adipocyte hypertrophy, CAP exposure increased adipose tissue inflammation and impacted the expression of clock genes in both LID and DIO. However, while CAP exposure affected insulin sensitivity in the liver and/or the skeletal muscle, CAP exposure had no effect on adipose tissue insulin signaling in either LID or DIO. In addition, CAP exposure alone shifted the diurnal abundance of adipose tissue macrophages and changed the 24 h clock gene expression. For instance, CAP exposure affected the number of macrophages and gene expression of clock in peritoneal macrophages collected at Zeitgeber (ZT) 3, 7, 11, 15 and 19 from mice maintained on a control diet and a 12h light/12h dark light cycle exposed for 30 days to HEPA-filtered air or CAP. Our results suggest that PM₂.₅ exposure exacerbates systemic insulin resistance in diet-induced obesity and light-induced dysrhythmia possibly by increasing adipose tissue inflammation and changes in circadian regulation of adipose tissue function.

**2311 Role of Gestational Respiratory Syncytial Virus and Ultrafine Particle Exposure on Maternal Infection and Offspring Outcomes**

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Pregnancy represents a unique period of immunomodulation designed to protect the developing fetus. Co-exposure to immune toxic pollutants, such as particles (UFPs), may serve as an additional susceptibility factor influencing the severity of respiratory infection and adverse outcomes in the progeny. For these reasons, respiratory infections represent a significant concern for pregnant women exposed to UFPs, yet data gauging the impact is limited. One such gap is susceptibility to respiratory syncytial virus (RSV), a common viral infection, and the role of UFP exposure in elevating severity of maternal infection. Therefore, the main objectives of the presented research were to identify whether pregnancy increases severity of RSV infection and if UFPs alter maternal immune status in a mouse inhalation exposure model. All protocols were approved by the Institutional Animal Care and Use Committee of Texas A&M University. Pregnant C57BL/6 mice were inoculated with a chimeric RSV strain (rA2-19F) or sham control on gestational day (GD) 14.5, or in age-matched non-pregnant female mice. The inoculum consisted of a 2,000 PFU dose of virus per nostril. Outcomes were evaluated 4 days post-infection (DPI) on GD 18.5, at which point tissues were collected and fetal crown-to-rump length (CRL) was assessed as a measure of development. Initial phenotypic results show similar weight gain trends among the sham and RSV-inoculated groups. Fetal CRLs were significantly lower on average for the progeny in the RSV exposure group compared to sham control. Histological examination of maternal lungs showed minor, focal inflammation in peribronchial and perivascular regions for sham groups, and mild to moderate scattered inflammation in peribronchial, perivascular, and alveolar regions for RSV groups. However, no differences were observed based upon pregnancy status. This could be due to low viral titer or narrow infection window. While RSV infection appears to impact developmental outcomes in offspring, the severity of maternal infection may be influenced by additional susceptibility factors. To investigate this question, pregnant C57BL/6 mice were exposed to a UFP mixture via whole body inhalation chambers from GD 0.5-14.5 for 6h/day to either a low dose (LD, 100 µg/m³) or a high dose (HD, 500 µg/m³). Lungs were processed into single cell suspensions and stained for CD4+ T cells expressing IFN-γ or IL-4, reflecting T helper (Th1) or Th2 re-
sponses, respectively. Preliminary data demonstrate a skewed Th2 ratio in the LD group. In the future, we plan to link our viral exposure model with a co-exposure to UFPs, to determine their impact on maternal respiratory infection.

2312 Impacts of Gestational Ozone Exposure on Placental Development and Vascularization

Gestational hypertension and preeclampsia are prevalent vascular disorders which significantly increase the risk for both prematurity birth as well as postnatal maternal cardiac risks. Numerous studies have shown correlations between higher air pollution levels and increased incidence of preeclampsia. However, current understanding of the mechanisms by which air pollution induce preeclampsia remains lacking. We have recently shown that inhalation of ozone during the early stages of placentation leads to maternal cardiovascular complications in a rodent model. The current study seeks to examine how ozone, as a constituent of air pollution, influences the development of the placenta at a cellular level. Using a cohort of ozone-exposed as well as a filtered air control Tie2GFP/FVBN mice (n=3), we employed an exposure paradigm of 1.0ppm ozone delivered at gestational day 10.5. The placentaion process then proceeded normally until gestational day 18.5, where tissue was harvested for analysis. Pooled placentas were digested for single cell RNA sequencing (10X Genomics). Differentially expressed genes indicated that mid-gestation exposure to 1.0ppm ozone resulted in a marked increase in cell types previously identified as inflammatory within the placenta. Notably, inflammatory and angiogenic pathway markers appear to be upregulated in ozone exposed animals when compared to filtered air controls. Further research is needed to determine whether pathways highlighted in the present study may serve as potential pharmaceutical targets for clinical treatment of preeclampsia or other gestational vascular disorders.

2313 Vesicular and Extravesicular Protein Signatures from the Airspaces of Ozone-Exposed Mice Reflect Muco-Inflammatory Disturbances
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Lung epithelial lining fluid (ELF) harbors a variety of proteins that influence homeostatic and stress responses in the airspaces. Exosomes, nano-sized extracellular vesicles, contain many proteins that vary in abundance and composition based on the prevailing conditions. Ozone causes inflammatory responses in the airspaces of experimental animals and humans. However, in ozone-exposed lung airspaces, the protein signatures in exosomes contained within the ELF remain poorly characterized. To explore this, we hypothesized that ozone triggers the release of inflammatory proteins from various cells that reflect ozone-induced tissue pathology. Accordingly, we sub-chronically exposed male Balb/c mice to 0.8ppm ozone or air and determined the protein profile of exosomes. A wide array of cellular types within the placenta. Notably, inflammatory and angiogenic pathway markers appear to be upregulated in ozone exposed animals when compared to filtered air controls. Further research is needed to determine whether pathways highlighted in the present study may serve as potential pharmaceutical targets for clinical treatment of preeclampsia or other gestational vascular disorders.

2314 Diesel Exhaust Particles Reduce Airway Epithelial Barrier Integrity through a Reduction of the Tight Junction Protein Tricellulin

Early life exposure to airborne particulate matter (PM) has been linked to the development of asthma. While changes to the expression of junctional proteins in lung epithelium have been seen in asthmatics, the impact of PM on their expression has received little attention. We investigated whether exposure to diesel exhaust particles (DEP), a major component of PM, would affect epithelial barrier function by reducing the expression of the tight junction protein Tricellulin. Standard reference material 2975 Diesel Particulate Matter (DEP; NIST) was suspended in culture media. Monolayers of the human bronchial epithelial cell line 16HBE14o- were grown on collagen coated Transwell inserts and exposed to 5 to 50 µg/cm^2 DEP for 6 to 24 hours. Changes in barrier function were assessed by measuring transepithelial electrical resistance (TEER) and permeability of 4 kDa FITC-Dextran. Tight junction protein levels were assessed by Western blotting of whole cell lysates. Neonatal Balb/c mice (postnatal day 3-5) were exposed to 255-889 µg/m^3 aerosolized DEP or filtered air for 2 hours per day for 5 consecutive days and sacrificed 2 weeks later. Lungs were collected, homogenized and analyzed by Western blot or RT-qPCR. Exposure to 25 and 50 µg/cm^2 DEP significantly reduced epithelial barrier function as measured by reduced TEER and increased permeability to 4 kDa FITC-Dextran at both 6 and 24 hours post exposure without inducing any detectable cytotoxicity. Co-treatment with 10 mM of the antioxidant N-Acetyl Cysteine had no effect on this DEP induced barrier dysfunction. These barrier changes coincided with a significant increase in inflammatory, xia histologic and permeability of 4 kDa FITC-Dextran measured by Western blot by six hours post exposure in 25 and 50 µg/cm^2 DEP exposed cells. Neonatal Balb/c mice (pnd 3-5) exposed to aerosolized DEP presented with a significant reduction in Tricellulin in the lung two weeks post exposure as measured by Western blot and RT-qPCR. Taken together, exposure to DEP caused a significant reduction in the expression of the tight junction protein Tricellulin. This reduction corresponds to a significant reduction in barrier function in vitro as measured by reduced TEER and increased permeability to 4 kDa FITC-Dextran without inducing cytotoxicity. Neonatal exposure to DEP caused a lasting reduction of Tricellulin at both the mRNA and protein level, suggesting early life exposure to DEP may cause a durable change in lung barrier structure and function.

2315 In Utero Ultrafine Particulate Matter Exposure Leads to Enhanced Murine Neonatal RSV Infection Severity

In utero exposure to particulate matter (PM) air pollution has been associated with increased lower respiratory tract infections (LRTIs) in infants. Despite the known sensitivity of the fetus to environmental pollutants and epidemiological evidence correlating prenatal PM exposure and LRTI morbidity, mechanisms of PM enhanced pathogenesis are relatively unexplored in immunologically immature populations. Moreover, the role of ultrafine particles (UFP, ≤ 0.1 µm) in the etiology of childhood respiratory disease are limited. To clarify the impact of in utero UFP exposure on LRTI morbidity, we exposed time-mated C57BL/6 mice to a low dose (LD) of PM (~100 µg/m^3), high dose (HD) of PM (~500 µg/m^3), or filtered air (FA) for 6-8 days from gestational day 0-18. At 5 days of age, offspring were challenged with culture media (sham control) or a chimeric strain,Passwords192F, of respiratory syncytial virus (RSV) previously shown to elicit an aberrant immune response similar to infant infection. At 3 days post infection (dpi), offspring were examined for viral load. At 9 dpi, offspring were examined for pulmonary inflammation, via histologic and bronchoalveolar lavage (BAL) fluid analysis, and pulmonary T cell profiles via flow cytometry. RSV successfully incited an inflammatory response in our model, and cytopathic effect in TCID50 assay indicated infectivity. Female weight disparities drove significant differences in weight gain across both sexes, particularly in the LD-RSV and FA-RSV groups. The inflammation by histology and viral load appear to have a positive, though not statistically significant, associated with increased PM exposure. Flow cytometry demonstrated a surprising thresholding effect in the LD-RSV group in the T regulatory cells and when calculating a Th2/Th1 ratio. This may suggest a yet unknown mechanism that activates that may induce the inflammatory cell numbers. In conclusion, UFPs appear to enhance neonatal RSV infection severity during in utero exposure, with mechanisms to be fully elucidated in future studies.

Virtual 2021 SOT Annual Meeting and ToxExpo
Epithelial cells have historically been the most researched cell type used to represent the human lung in in vitro models of disease; however, recent technology has increased their utility for predicting adverse human health outcomes. Novel complex and sensitive platforms can evaluate the effects of combustion-derived pollutants, including diesel exhaust particulates (DEP) and woodsmoke (WS), on human lung cellular components, another (PM) known to be responsible for pre-mature morbidities and co-morbidities. Using Transwell inserts, we developed a multicellular model of the alveolar capillary region recapitulated by human lung epithelial cells (H441s), lung fibroblasts (IMR-90s), and lung microvascular endothelial cells (HULECs). As the first line of defense, epithelial cells are plated on the apical surface of a Transwell insert, with fibroblasts on the basolateral surface, and endothelial cells in the underlying well. Direct exposure to DEP and WS in the apical compartment recapitulates direct epithelial cell exposure in the human lung and allows us to quantify changes in each cell type as a result of the epithelial cell response. Epithelial barrier integrity and viability is maintained up to 24 hours after PM exposure. However, changes to endothelial cell gene and protein expression support the presence of cell signaling changes associated with epithelial cell response in the absence of cell death or translocation of PM through the epithelial barrier. Further investigation reveals significant gene expression changes as early as six hours (DEP) or eight hours (WS), following exposure for genes involved in redox cycling (PRDX1, PRDX6, TXN, GSR) and oxidative-stress-associated autophagy (SQSTM1). This was confirmed by significant changes to proteins involved in protection against oxidative stress (HMOX-1), glutathione synthesis (GLCM, GCLC), oxidative-stress-associated autophagy (SQSTM1), and oxidant stress to elucidate specific drivers of DEP and WS toxicity, assess changes in kinase activation, and reveal the specific secreted factors generated by epithelial cells responsible for endothelial cell response. Models like these are necessary to increase the value of in vitro testing as a means of reducing cost and animal use associated with in vivo studies while maintaining the complexity of the lung microenvironment.

**2317 Identifying the Molecular Mechanisms of Air Pollution-Induced Cardiovascular Disease**

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An estimated 3.5 million people die annually from air-pollution-induced cardiovascular disease (API-CVD). API-thrombosis (API-T) is a common precursor of these cardiovascular mortalities; however, the molecular mechanisms driving API-T remain unclear. To identify these mechanisms, we developed a novel in vitro model that mimics the in vivo exposure scenario and that represents the interaction of the respiratory and cardiovascular systems. This alveolar-capillary region (ACR) is organotypic with human alveolar-like epithelial cells (H441), human lung fibroblasts, and human lung microvascular endothelial cells (HULEC). We hypothesized that air pollution exposure of H441 cells would induce endothelial dysfunction in the HULEC, initiating the onset of a pro-thrombotic state in the ACR. To test this, we exposed confluent monolayers of H441 cells to the ubiquitous air pollutant, diesel exhaust particles (DEP), and investigated the effect of this trans-alveolar DEP exposure on the underlying HULEC. TA-DEP exposure activated the oxidative stress-responsive transcription factor, nuclear factor erythroid-2 related factor (NRF2), and induced its antioxidative targets, heme oxygenase 1 (HMOX-1) and NAD/P(N)H dehydrogenase [quinone] 1 (NQO1) in the HULEC. Regardless of this antioxidative response, increased reactive oxygen species (ROS) and decreased glutathione redox potential occurred in the HULEC. Concurrently, we observed decreased expression of multiple endothelial fibrinolytic and anti-coagulant genes in the HULEC, and decreased expression of the procoagulant gene, coagulation factor III (F3). These pro-thrombotic and antioxidant gene expression changes were recapitulated in primary human lung microvascular endothelial cells. Collectively, these data suggest that DEP exposure induces an endothelial antioxidant response that is unable to prevent accumulation of ROS, resulting in endothelial redox dysfunction. In addition, these data suggest that pro-thrombotic activation may occur in the capillary beds of the ACR upon DEP exposure. Endothelial redox dysfunction and pro-thrombotic activation in the ACR can lead to systemic pro-thrombotic activation, resulting in API-T and ultimately API-CVD. Thus, this in vitro model is a novel tool to identify the molecular targets and effects of inhaled toxicants and can help prevent air pollution-induced mortalities. Does not reflect US EPA policy.
Air pollution's toxicological effects on human health is one of the main public health concerns in highly populated cities. Particulate matter less than 2.5 microns (PM$_{2.5}$), one of the main constituents of the air pollution, have been associated to diverse adverse effects such as pulmonary damage, lung cancer, cardiovascular diseases and also metabolic diseases. Despite the associations, the main mechanisms leading to the metabolic diseases from PM$_{2.5}$ exposure are not completely understood. Different parameters such as insulin resistance, basal glucose increase, and the homeostatic model assessment for insulin resistance index (HOMA-IR) have been directly related to metabolic diseases. Recently, the activation of the Rho-A/Rho kinases pathway induced by the exposure of PM$_{2.5}$ can lead to the inhibition of the insulin pathway (insulin resistance). Our work aims to determine the presence of insulin resistance from the inhalatory subchronical exposure of Sprague Dawley rats to PM$_{2.5}$, with and without a concomitant exposure to fructose (in the drinking water). Four exposure groups were used in this study, filtered air, PM$_{2.5}$, filtered air and fructose, and PM$_{2.5}$ and fructose. Inhalatory exposure to Mexico City PM$_{2.5}$ was performed during eight weeks between October to December 2020. Until now general parameters as height, weight, water consumption, and body mass index were determined. A higher increase in the height, weight, and body mass index was observed in the individuals of the exposed to fructose. A higher increase in the height, weight, and body mass index were observed in the individuals of the exposed to fructose group. A significant increase in glucose levels were observed in the rats exposed to PM$_{2.5}$ and fructose up to week six of exposure. These findings in the exposed animals to PM$_{2.5}$ and fructose can be associated to a dysregulation in metabolism and possibly lead to insulin resistance, which will be confirmed by HOMA-IR and insulin levels. In addition, protein levels of members of the insulin pathway and the activation of the Rho-A/Rho kinases pathway are necessary to determine their possible relationship with insulin resistance, the metabolic diseases and the exposure to PM$_{2.5}$.

**2320 Increase in Glucose Levels Induced by Subchronical Co-exposure to Mexico City PM$_{2.5}$ and Fructose in Sprague Dawley Rats**


**2322 Atmospheric 4-Nitrocatechol Exposure Causes Mitochondrial Dysfunction and Induce Apoptosis in the Lung BEAS-2B Cells**

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Biomass burning results in the emission of 10-15 Tg yr$^{-1}$ of organic carbon (OC) into the atmosphere globally. The UV-light absorbing OC component contains a group of poorly characterized nitrated aromatic compounds (NACs). These NACs are important secondary organic aerosol (SOA) products of urban bio-mass burning aerosol (BBA) involving nitrogen oxides (NOx) as key reactants. The present work determines the inhalation toxicity profile of 4-nitrocatechol, a predominant precursor of BBA, in BEAS-2B cells (normal human bronchial epithelial cells). Inhibitory concentration-50 (IC$_{50}$) in BEAS-2B cells was found to be 1 µg mL$^{-1}$ at 24-hour and 4 µg mL$^{-1}$ at 48-hour of exposure. LDH release assay revealed ~ 80% of cellular death after exposure to 100-200 µg mL$^{-1}$ of aerosol. Flow cytometric analysis through Annexin-V/ Propidium Iodide (PI) staining of treated cell population revealed ~ 50% of BEAS-2B in late apoptosis after 24-hour when treated with 200 µg mL$^{-1}$ of aerosol. This phenomenon was confirmed through appearance of apoptotic bodies in aerosol exposed cells between 12-24-hour using Calcein-AM/ PI staining under the fluorescence microscope. Increase in Carboxy-DCFH staining in treated cells revealed buildup of reactive oxygen species (ROS) inside the treated cells. A decreasing tetramethyl rhodamine (TMRM) signal was observed with increasing time of 4-nitrocatechol exposure, indicating the collapse of mitochondrial membrane potential in the BEAS-2B cells. Enhanced mitochondrial-specific signals using Mito-Sox (super-oxide indicator) and Mito-PY1 (hydrogen peroxide indicator) confirmed that dysregulation of mitochondrial function resulted in cellular death through apoptosis. The study highlights the need to control emission rates of 4-nitrocatechol into the atmosphere due to its potential inhalation toxicity following acute exposure. Its atmospheric release can be controlled through the regulations of NOx levels and decreasing instances of biomass and wildfire burning.
2323a High-Fructose Diet Affects Cardiovascular Function after a Single Wood Smoke Exposure in Adult Wistar-Kyoto Rats

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Wildfires are extreme events that have significant potential to harm both healthy individuals and those with underlying conditions like cardiovascular disease. We previously demonstrated that exposure to a single episode of wildfire or wood smoke can alter homeostatic function and worsen cardiovascular responses in healthy animals. However, it is not well established how diet affects the cardiovascular response to wildfire smoke. High-fructose (HF) intake has been associated with the development of obesity, inflammation, increased oxidative stress, and autonomic imbalance. This study examines the effects of a HF (30% fructose, 0% sucrose) diet on cardiovascular responses to wood smoke (WS) in Wistar-Kyoto rats. We hypothesized that a HF diet would alter basic metabolism, body composition, and hemodynamic function of the heart, and worsen the subsequent response to WS. Eight-week-old Wistar Kyoto rats were placed on either a 30% HF or normal diet (ND) for seven weeks. Body composition was measured every three weeks for fat, lean, and fluid compartment percentages, and animal resting energy metabolism was analyzed using an indirect calorimeter. Following the respective 7-week diet, each group was exposed to 5 mg/m³ of wildfire or filtered air (FA) for one hour. 24 hours after the exposure, rats were anesthetized, and cardiac function was determined with echocardiography. There were no significant changes in body composition in HF rats compared to the control. Respiratory exchange ratio significantly decreased in the HF diet compared to the control in both the light and dark cycle. HF rats exposed to WS had a significantly longer isovolumic relaxation time when compared to ND exposed to WS. Additionally, HF exposed to WS had a higher left ventricular myocardial performance (MPI) than ND exposed to WS. Exposure to WS caused standard deviation between NN intervals (SDNN) to increase and root mean square of successive differences (RMSDD) to decrease in ND rats, on the other hand, HF rats exposed to WS had an increase in the latter and showed a trend towards increased SDNN. There were no significant differences in frequency domain parameters. These results indicate that a HF diet alters baseline cardiovascular function even in the absence of body composition changes in Wistar Kyoto rats and suggests that diet can predispose the cardiovascular system to adverse effects during and after a single one-hour smoke exposure. Disclaimer: This abstract does not necessarily reflect US EPA policy.

2324 Retrospective Evaluation of the Acute Fish Toxicity Test for Pesticide Registration


The acute fish toxicity test is used to assess the potential risk of substances to aquatic organisms. In the United States (U.S.), the test is typically conducted in three different fish species: a cold and a warm freshwater species and a marine/estuarine species. Therefore, three separate acute fish toxicity tests are conducted for each test chemical with potential for exposure to 200 or more fish per chemical. We conducted a retrospective analysis of data submitted to the U.S. Environmental Protection Agency (EPA) to support pesticide registrations to determine whether reducing the number of species tested would impact the ability of pesticide risk assessments to identify and characterize potential risks to fish. Lethal concentration 50% (LC50) values and experimental details were extracted and curated from 762 acute fish toxicity studies submitted to EPA for pesticide registrations. From this data set, 87 substances had data from studies in three species that were considered acceptable based on defined criteria. Data were analyzed to determine any trends among species in terms of relative differences in acute toxicity LC50 values. Where a difference could be detected, coldwater species were most often the most sensitive species (26/45); for the remaining 42 substances there was no clear difference in species sensitivities. These results suggest that it may be possible to use data from as few as one species, thereby substantially reducing the number of fish required while still meeting risk protection goals. This project was funded in whole or in part with federal funds from the NIH, NIH under Contract No. HHSN272201500010C.

2325 Comparative Toxicity of Model Fish and Amphibian to Trifloxystrobin Early Life-Stage Exposures

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Chemical exposures that lead to adverse developmental effects of individuals in could result in significant population-level impacts. Current risk assessment practices use early life stage fish data when information for larval amphibians is not available. While fish are sufficiently sensitive to a range of chemicals for juvenile life stage evaluation (e.g., developmental delay), they do not have greater impacts on subsequent amphibian life stages that are not represented by fish. As pesticide exposure contributes to amphibian population declines, it is important to understand the uncertainties of species surrogacy in risk assessments aimed at protecting amphibians. Strobilurin fungicides are an economically important class of pesticides designed to inhibit the mitochondrial respiratory chain ultimately interfering with ATP production. Trifloxystrobin (TX), a widely applied strobilurin, is frequently detected in aquatic systems, often at levels known to cause sub-lethal toxicity in a range of species. In this study we conducted the first side by side comparison of 10-day early life stage exposures conducted on zebrafish (Danio rerio), fathead minnow (Pimephales promelas), and African clawed-frog (Xenopus laevis) to TX to determine if growth, development, and gene expression changes were similarly altered across species. Mortality data suggests that acute toxicity values derived from D. rerio are not ideal surrogates for lethality in X. laevis. While a delay in hatch was observed in both fish species, weight and length were only reduced in P. promelas. A dose-dependent decrease in weight and a developmental delay were observed in X. laevis. Here, we also report the first investigation of gene expression changes in P. promelas and an amphibian species following exposure to a strobilurin. Tissues collected from exposed individuals were used to compare differential gene expression on targets related to endocrine signaling, oxidative and general stress responses and apoptosis. Gene expression patterns suggest that biological pathways are perturbed by TX exposure in a species-specific manner. Future studies will examine the potential of the observed changes in gene expression to be predictive of more population-relevant endpoints, such as time to metamorphosis.

2326 Antivirals in the Environment: Potential Contributors to Antimicrobial Resistance

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Antiviral pharmaceutical compounds are used as treatment and prophylaxis for long-term and acute viral infections. Antivirals can enter the aquatic environment in human waste or as the result of pharmaceutical manufacturing. Antivirals have been detected in wastewater, surface water, and ground water throughout the world. Current wastewater treatment processes are often insufficient to remove many antivirals from wastewater. Substantial evidence points to overtuse of antibiotics and presence of antibiotics in the aquatic environment as key aspects contributing to the rise in antibiotic resistance throughout the world. However, other major pharmaceutical classes including antivirals can have antibacterial effects and may also play a role in the development and proliferation of antimicrobial resistance. Using standard culture methods and optical density to monitor bacterial growth over 48 hours, we investigated the antibacterial capacity of 12 antivirals on E. coli and B. subtilis in concentrations from 0.01 µg/ml to 100 µg/ml. Antiviral compounds tested included zidovudine, stavudine, lamivudine, didanosine, tenofovir, nevirapine, abacavir, acyclovir, efavirenz, raltegravir, dolutegravir, and favipiravir. When bacteria were treated with individual antivirals, seven of the antivirals tested exerted antibacterial effects on E. coli at concentrations less than 100 µg/ml; three of the antivirals tested exerted antibacterial effects on B. subtilis in concentrations less than 100 µg/ml. Both E. coli and B. subtilis could quickly become resistant to antibacterial antivirals. Resistance to one select antiviral often conferred cross-resistance to at least two other antivirals when bacteria were challenged. Furthermore, resistance to selected antivirals was observed in antiviral-resistant E. coli and B. subtilis. Antiviral-resistant bacteria are cross-resistant to a range of structurally distinct antivirals and antibiotics, suggesting that mechanisms of antimicrobial resistance that develop due to antiviral exposure may have similarities despite the structural diversity of parental chemotypes. The presence of antivirals in the aquatic environment is a significant public health concern, as antivirals have the potential to contribute to the global antimicrobial resistance crisis.
A systematic evidence mapping (SEM) project was proposed to SOT undergraduate students in the Undergraduate Diversity Program. The purpose of this project was to expose and train students to an emerging tool, while also involving them throughout the SEM process. Similar to the SOT 2020 UDP One-Health activities, this project investigates how shark exposure to anthropogenic pollutants (e.g. persistent organic pollutants, metals, microplastics, etc.) impact both shark and human health. This is a timely topic as other studies have not evaluated the toxicological implications of pollutant exposures within these globally consumed organisms. This SEM utilizes a Population, Exposure, Outcome (PEO) statement to answer the following primary question: How are global shark populations affected by anthropogenic pollutants? These mapping scopes helped develop the search strategy, which included an extensive list of pollutant exposures and considered all shark species. To screen studies and ensure each reviewer independently reviewed each article, the online platform called Covidence was used. A total of 1,148 articles were imported from database searches. Of the studies screened, there are both geographical and species-specific trends relating to pollutant exposure, with gender, body indices, and age factoring into reported concentration values. Several studies investigated gestational relationships with a focus on the embryonic transfer of metals. More generally, mercury and methylmercury were frequently associated with other studies investigating other metals. There were organ specific distributions, but the consequent health impacts depended upon which tissues were sampled. These findings demonstrate a need to understand the role of pollutant burden in sharks, while also identifying which shark organs are relevant for human health. This project demonstrates how SEMs can address broad topics, while also signifying their utility in toxicological subdisciplines.

Toxicological safety data for compounds used in both industrial and domestic settings is necessary to characterize and mitigate risk to both human and ecological health, yet is deficient for 86% of existing chemicals. These deficiencies, coupled with the significant time and monetary costs of traditional toxicity testing, have prompted a shift away from historically used methods towards those that reduce the number of animals, leverage existing data, and implement high throughput screening (HTS) techniques. Due to the high fecundity, rapid development and sensitivity, current in vivo screening measures commonly utilize embryonic fish as toxicity models. Zebrasfish (Danio rerio) in particular share a high degree of genetic similarity with humans (over 70% of human genes have a zebrafish ortholog), making them a popular model for both human health and toxicological studies including the fish embryo toxicity (FET) test. While the FET test typically exhibits a high level of correlation with the traditional Acute Fish Toxicity test (AFT), there is evidence that FET demonstrates weaker toxicity than AFT for some compounds. Previous research in our lab has shown that larval fish exposed to the anti-histamine diphenhydramine (DPH) demonstrate increased mortality, uptake, and behavioral toxicity than their embryonic counterparts. However, whether dispositional and/or molecular initiation events influenced such age-specific differences were not identified. The current research targets this literature gap by examining changes in proteome expression between larval fish exposed to DPH at 4- and 10-days post fertilization (dpf) at sublethal concentrations (1/10 LC50). Over 111 proteins were identified (1% FDR) and grouped based on functional and expression-specific differences in the identity, proportion, and function of identified proteins.

Parathion is an organophosphate (OP) pesticide used worldwide which exhibits the characteristic OP mechanism of toxicity, the inhibition of acetylcholinesterase causing hyper-excitation of the cholinergic nervous system. Given that environmental release of parathion-based pesticides is the standard use case, we were interested in determining if sunlight transformed parathion and therefore affected toxicity. Thus, we tested the null hypothesis that parathion is not transformed by sunlight and has no change in toxicity. Ethyl-parathion dissolved in water was exposed in replicated experiments to direct sunlight for 0 (dark control), 1, 2, 3 and 4 hours where singlet oxygen species (O1(D)) formation and associated cell-free assessment of DNA damage through degradation of guanine nucleotides. Additionally, superoxide anion radical (O(-)2-) radical increased through time, likely via type-II photophysical mechanisms. Genotoxic properties of sunlight-degraded parathion was suggested by increased photo-oxidative degradation of 8-hydroxy-2-deoxyguanosine (a guanine base of DNA) with increasing sunlight-exposure time which was measured at 260nm. Our result showed that up to 52% degradation of guanine at the maximum exposure to sunlight (4 hours). The generation of O2- was monitored by recording the photosensitized reduction of NBT (Nitro blue tetrazolium chloride) to nitroblue diformazan (NBF) spectrophotometrically at 560 nm. The generation of O2- by parathion was further confirmed by specific quencher superoxide dismutase (SOD) and our result showed that 62% inhibition by 4 hours of sunlight exposure. Our results further showed the photogenotoxic and photocytotoxic property which were assessed photochemical by chemically at 233nm. Our results suggest potential mechanisms by which dissolved parathion in sunlight could result in photosensitization as indicated by increased readouts of the wavelength where parathion was excited to its reactive triplet state which lead to the formation of ROS. The reaction of these products which degrade DNA (guanine base) and increased linoleic acid photo peroxidation provide screening-level evidence for photogenotoxic and photocytotoxic property of parathion in sunlight.
human whole blood and that prenatal Pb exposure’s alteration of gene-specific 5mC and 5hmC levels in blood are stable into adolescence, providing evidence to consider 5hmC as a regulatory response mechanism to environmental exposures.

2331 Mitochondrial Dysfunction Induces Epigenetic Dysregulation by H3K27 Hyperacetylation to Perturb Active Enhancers in Parkinson’s Disease

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Genetic mutations explain only 10–15% of cases of Parkinson’s disease (PD), whereas an environmental component has been implicated in the etiopathogenesis underlying the remaining majority of PD cases. But regardless of where the triggers for the onset of familial and sporadic PD fall on the gene-environment axis, mitochondrial dysfunction emerges as a common mediator of dopaminergic neuronal degeneration. Herein, we employ a multidisciplinary approach to convincingly demonstrate that neurotoxicant exposure- and TFAM knockout-driven mitochondrial dysfunction share a common mechanism of epigenetic dysregulation. Under both scenarios, Lysine 27 acetylation of likely variant H3.3 (H3.K27ac) increased in dopaminergic neuronal models of PD, thereby opening that region to active enhancer activity via H3K27ac hyperacetylation. These vulnerable epigenomic loci represent potential transcription factor motifs for PD pathogenesis. We further confirmed that mitochondrial dysfunction induced H3K27ac during neurodegeneration in ex vivo models of PD. Our exciting results reveal an axis for PD pathogenesis comprising ‘exposure/mutation-mitochondrial dysfunction-metabolism-H3K27ac-transcriptome’. Collectively, the novel mechanistic insights presented here link mitochondrial dysfunction to epigenetic transcriptional regulation in dopaminergic degeneration as well as offer potential new epigenetic intervention strategies for PD. Support: R01ES072745, R01ES026892, R01NS100090 and R01NS088206, Eugene and Linda Lloyd Endowment and Arambut Endowment.

2332 Lead Exposure during Neural Differentiation: Effects on piRNA Expression and Implications for Neurodevelopment


In addition to the classically studied epigenetic mechanisms of DNA methylation and histone modifications, small, non-coding RNAs serve as an epigenetic link between environmental exposures and adverse health outcomes. PIWI-interacting RNA (piRNA) associate with PIWI proteins to direct the silencing of transposons via DNA methylation, and this regulation is a key component of epigenetic programming. The piRNA system is thought to lie upstream of the germline, our work in mouse and human tissues has identified sex- and tissue-specific expression of piRNA and PIWI proteins in the soma. Our group has demonstrated the piRNA system is expressed to a significant degree in the mouse and human brain, suggesting it may have important roles in epigenetic programming during neurodevelopment. In early developmental human tissues (gestational day 90-105), PIWIL1 and 2 mRNA, while expressed to a lesser degree than in the germline, showed some of the highest relative expression in the brain when normalized to the gonads. The relative expression of PIWIL3 and 4 in the brain exceeded that of the germline, with 182% and 294% normalized to the gonads, respectively. Our group has previously demonstrated that perinatal lead (Pb) exposure disrupts retrotransposons within the mouse brain, with 3.86%, 2.83%, and 1.77% less DNA methylation at intracisternal a particles (IAPs) 110, 236, and 506, respectively. We have also found LINE-1 retrotransposons to be hypomethylated in the blood of adolescents who experienced prenatal Pb exposure. Due to the Pb-associated disruption of transposon methylation, we are now studying to what degree disruption of the piRNA system by developmental Pb exposure contributes to the observed aberrant DNA methylation. We are investigating this relationship first within the SH-SY5Y neuroblastoma cell model, with a focus on changes in piRNA and PIWIL mRNA expression, DNA methylation, and changes in morphological and molecular markers of neural differentiation. We are conducting this work in the hope of solidifying the connection between early life exposures and adverse neurocognitive outcomes that persist throughout the life course in order to better inform intervention and therapeutic strategies.

2333 Quantitative Dose-Response of Liver MicroRNA after Furan Exposure in Rodent Liver, Blood, and Cell Culture

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Gene expression alterations in the liver due to environmental chemical exposure can indicate early and dose-responsive measures of later adverse outcomes. Measuring these alterations in short-term models may help reduce the cost, time, and uncertainty associated with assessing chemical hazard and risk. Recent studies in our lab have demonstrated that microRNAs (miRNAs) may similarly indicate chemical potency and mode-of-action associated with health outcomes of regulatory concern. Here, we examined the miRNA response to furan, a rodent hepatocarcinogen with a postulated mode of action of chronic cytotoxicity followed by regenerative proliferation. Previous gene expression measurements of short-term furan exposure in B6C3F1 mice were highly predictive of published cancer bioassay point of departure values. Using liver and blood samples from these same 3-week exposure studies to 0, 1, 2, 4 and 8 mg/kg body weight per day furan, we evaluated miRNA-based benchmark doses (BMDs) which were estimated based on small RNA sequencing and Nanostring (miRNA and gene expression) measurements. In addition, we examined miRNA expression changes in furan-treated female B6C3F1 primary hepatocytes for comparison with in vivo results. In the mouse liver, miRNAs were significantly altered with carcinogenic doses of furan and were implicated in proliferation, apoptosis, cell cycle regulation, and other cancer signaling networks. When mapped to altered mRNA, these miRNAs were identified to ATM and p53-mediated pathways and showed a robust dose response for 43 mRNA (BMD range 1.0-6.7, AVG 2.1) and 196 mRNA (BMD range <0.1-7.7, AVG 3.0). These results suggest that miRNA are more sensitive biomarkers of furan exposure and hepatotoxicity than mRNA and may be more reflective of the 2-year cancer bioassays (BMDs for HCA 2.3, HCC 2.6). Some dose-responsive miRNA were also altered in the blood (e.g., miR-676, miR-532, miR-34a, miR-183) and in primary hepatocytes (miR-34a). Overall, these results indicate mechanistic involvement of miRNA in furan carcinogenicity and provide evidence of their potential utility as accessible, dose-responsive biomarkers of chemical-mediated disease outcome. This abstract does not necessarily reflect US EPA policy. Mention of trade names is not an endorsement or recommendation for use.
Programming Liver Disease from Developmental Exposure to Cadmium

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Non-alcoholic fatty liver disease (NAFLD) is a public health crisis, costing $103 billion/year and affecting ~30% of the US population. It is a progressive disease characterized by fat accumulation, inflammation, and liver scarring. Susceptibility to NAFLD is programmed by the developmental environment, including exposure to the toxic heavy metal cadmium (Cd), which is one of the top ten toxicants of public health concern according to the World Health Organization. Epigenetic mechanisms have been proposed as mediators of this developmental programming, but their exact nature and how they impact adult disease is poorly understood. Our team proposes that exposure to Cd in early life alters the epigenetic regulation of imprinted genes, which are expressed from only one allele and transcriptionally co-regulated as part of an Imprinted Gene Network (IGN), leading to NAFLD. In support of this hypothesis, gene set enrichment analysis of the IGN showed that the IGN is enriched for functions associated with liver disease. Using a cultured hepatocyte cell line, we demonstrated a functional link between activation of the IGN and NAFLD-related phenotypes. Now, using a unique, hybrid mouse model, we have been testing the importance of the IGN in the programming of NAFLD by developmental exposure to Cd and determining the underlying epigenetic causes of IGN dysregulation. To date, we have shown that Cd exposure during development leads to striking liver and body weight phenotypes. Using qPCR analysis, we have shown that IGN genes are differentially expressed at birth but are significantly differentially expressed between exposure and control groups at three weeks of age. Genes important to lipid accumulation and fibrosis are significantly increased in expression at three weeks of age in the Cd exposed groups. Also, histological analyses are indicative of a NAFLD phenotype at three weeks of age. Our hypothesis is that Cd activation is caused by a relaxation of imprinting driven by changes in DNA methylation. Successful completion of this work may identify a novel role of the IGN as a bridge between the developmental environment and later life disease and reveal important targets for potential therapeutic interventions.

Regulation of Autophagy during Pentachlorophenol Exposure in In Vitro Study

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Pentachlorophenol (PCP), an organochlorine pesticide, has been primarily used as a wood preservative in the U.S. However, it is ubiquitous in the environment because of its widespread use in agricultural, domestic, and industrial applications. Due to its carcinogenic activity, the use of PCP was restricted by the US EPA. Since it is an environmental toxicant, PCP is easily absorbed through the skin and lungs. Chronic exposure leads to severe lung and liver toxicity in humans. There are few reports which demonstrate PCP-mediated increase in inflammatory responses using murine and cellular study models. However, the molecular mechanisms associated with PCP-mediated inflammation are yet to be explored. The role of autophagy in regulating inflammatory mediators including pattern recognition receptors, cytokines, and chemokines has been well studied. We therefore hypothesized that the autophagy process plays an important role in PCP-mediated regulation of inflammatory responses in human adenocarcinoma cells (A549) and human liver carcinoma cells (HepG2) cells. In our study models, PCP-exposure induced several autophagy proteins (Becn1, Atg5, Atg12, Atg16). Earlier studies support the role of autophagic changes, such as DNA methylation and histone modifications, in the regulation of autophagy. In this regard, we observed dysregulated global and specific DNA methylation at CGP promoter sites of Becn1-1 and LC3B in A549 and HepG2 cells. With regards to histone modifications, we focused on three chromatin marks-trimethylation of histone 3 lysine 4 (H3K24me3); and the trimethylation of histone 3 at Lys 4 (H3K4me3) and Lys 9 (H3K9me3). Our results demonstrated an increase in histone modification associated with active gene transcription (H3K4me3) in PCP-exposed A549 cells, whereas we observed dysregulated H3K9me3 and H4K20me3 in PCP-challenged A549 and HepG2 cells. Interestingly, both H3K9me3 and H4K20me3 are associated with gene repression. We also observed a upregulation of methyltransferases (SET1, ESET, SUV2D1H1 in HepG2 cells. These enzymes are responsible for histone modifications, whereas downregulation activity in PCP-challenge A549 cells correlated with the epigenetic markers -H3K9me3 and H4K20me3, respectively. Further work is in progress for deeper understanding about the role and regulation of epigenetic signatures in regulating autophagy related proteins in our study models.

Broad Utility of the FirePlex Multiplexed Assay Technology in Toxicology

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Novel biomarkers of drug-induced organ toxicity are needed to help deliver safer medications with a wider therapeutic window. Multiplexed assays have the potential to expedite biomarker discovery by allowing the interrogation of changes in proteins or their up-stream regulators in human and experimental toxicology research settings. FirePlex is a novel multiplexed assay technology which has been developed to detect and quantify both proteins and micro-RNAs. To enable higher throughput, FirePlex has been adopted to interface with laboratory automation using a two-step, no wash assay workflow and miniaturization. Three FirePlex human toxicology multiplexed panels were developed: kidney, cardiac and liver. These protein biomarker-based panels were tested with human serum and urine samples from healthy individuals as well as chronic kidney disease, cardiac heart failure and non-alcoholic steatohepatitis patients, respectively. Significant inter-individual variations in all candidate micro-RNA biomarker sets were observed. DNA methylation in micro-RNA candidate urinary biomarkers of drug (Ampthomeric B)-induced nephrotoxicity in rats and mice. Urine was collected from drug-treated animals and subsequently analyzed using the FirePlex rodent miRNA multiplexed assay to screen approximately 400 candidate micro-RNA biomarkers. FirePlex 5-plex mouse kidney toxicity panel consisting of known protein biomarkers of kidney injury was used to monitor for drug-induced kidney tissue injury. Several micro-RNAs were found to be upregulated in both rodent models in a temporally distinct pattern consistent with microscopic kidney lesions. These results demonstrated broad utilities for FirePlex multiplexed assay technologies in different scenarios of clinical and nonclinical toxicology.

Alterations in DNA Methylation Patterns among African Americans and Whites Conferring Risk to Breast Cancer Susceptibility


Breast cancer is one of the most common cancer types being diagnosed among women. Recent studies showed that there is a well-documented disparity in breast cancer survival, which is stratified by race, socioeconomic status, education, preventive care, and access to health insurance. In this study, we assessed the differences in the DNA methylation (DNAm) pattern for six high-risk breast cancer susceptibility genes among African American (AA) (n=160) and White (n=578) participant samples. We have downloaded the methylation datasets from the primary solid tumors of breast invasive carcinoma from TCGA database for AAs and Whites from the GDC Data Portal using R package TCGAbiolinks. Differential methylation analysis using TCGAanalyzer_DMR funct was performed to identify differentially methylated regions (DMRs) at a mean methylation cut-off of 0.25 and at a significant p-value <0.01. A list of CpGs that are significantly differentially methylated between AAs and whites were subjected to pathway analysis using the Ingenuity pathway analysis (IPA) core pathway and upstream regulator analysis (Quagen Ingenuity Systems). Gene ontology (GO) enrichment for the significant CpGs was analyzed using the gemeth function in missMethyl package. We identified two CpGs in BRCA1 (cg20760603, cg21253966), seven CpGs in BRCA2 (cg08157964, cg25753010, cg27253386, cg00031759, cg06935361, cg05137335, cg08193369), nine CpGs in CDH1 (cg26508465, cg08615685, cg16739895, cg10331337, cg05785947, cg18578229, cg09220040, cg43998833, cg23998635), five CpG site in PALB2 (cg10313337, cg05785947), and eleven CpGs in PALB2 (cg26508465, cg08615685, cg16739895, cg10331337, cg05785947, cg18578229, cg09220040, cg43998833, cg23998635), ten CpG site in PALB2 (cg10313337, cg05785947, cg18578229, cg09220040, cg43998833, cg23998635), and ten CpG site in PALB2 (cg10313337, cg05785947, cg18578229, cg09220040, cg43998833, cg23998635), respectively. Further work is in progress for deeper understanding about the significance of environmental exposures to elucidate the effect of various chemicals on germline epigenetics and how paternal exposure may impact the health of future generations.
2339 Health Impact of E-cigarettes: An Updated Perspective on Characteristics, Compositions, and Toxicological Effects of E-cigarettes for the Most Common Flavors: Tobacco and Menthol/Mint

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In 2007, Electronic cigarettes, also known as vapes, vaporizers or vape pens entered the US market as presumed safer alternative over conventional combustible cigarettes. To target both adults and children, a huge diversity of 8,000 e-cigarette flavours are available in the market. Celebrity endorsements, false religious beliefs, deliberate addition of flavouring agents are encouraging e-cigarette use and product publicity. In this study, we have exhaustively analysed the e-cigarette products in the current market and listed the products based on the most common flavours (menthol, mint and tobacco). To assess the safety of these flavours, we also compiled and critically examined the current toxicology data on the exposure in rodent models and in vitro human cell culture. We observed that the current market share is highest for tobacco (more than half) between the three flavours. The current toxicology data demonstrates that usage of tobacco and/or menthol/mint flavouring agents in e-cigarettes leads to increased inflammatory cytokines, airway flow resistance and cytotoxicity. As the new federal regulation still allows the usage of tobacco and menthol/mint flavouring agents (while banning other fruity flavours), the preference and prevalence of tobacco and menthol-flavoured e-cigarette will finally result in enhancing their usage and thus contradicts the expected decline in e-cigarette usage.

2340 Facility Qualification and Study Audits: 2020 and Beyond

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Since Early 2020, working practices have been revolutionized due COVID-19. These changes have particularly impacted travel and consequently the processes for in person site qualifications and study monitoring. Performing a through audit of a new partner is important to ensure that the CRO is capable of performing the work and is also an excellent platform to start to build/ foster relationships with the CRO. The auditing process can be detailed and extensive requiring the participation of many personnel. This article will look at the key steps to optimize the auditing/review process in these exceptional times. This optimization starts with the client reaching out to the CRO and arranging an audit at a mutually acceptable time. Followed by identifying the site lead, they will be your main point of contact, this person will provide requested documents, organize CRO personnel etc. These documents will be placed in secure virtual rooms for the reviewer ahead of time. This allows for the detailed review prior to meeting the facility staff, so when virtually face to face the discussions can be focused and direct, addressing any potential issues quickly and to the point. Using a variety of platforms meetings can be held in real time with facility staff. With careful planning and communication these can be efficient and enabling objectives to be met. This process is still in line with SQA and allows clients to complete audits from their office/home and thereby addressing current travel restrictions. Essentially the only difference is the onsite face to face, and data is placed in a virtual room as opposed to a board room, by using the available tools, data rooms, meeting rooms, online meeting platforms in a timely efficient manner. The key factor here is effective and proactive communication between the Sponsor and the CRO. Indeed such practices can be carried forward beyond 2020, as by using these mediums the need for foreign/domestic travel is eliminated, thereby increasing the efficiency of the audit/monitoring.

2341 International Regulatory Needs for Acute Toxicity Data

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Chemical regulatory authorities around the world consider acute systemic toxicity data to substantiate safety assessments, and for many regulations these data are typically used to develop product hazard labels for consumer or worker protection and to assess risks from acute exposure to chemicals. Other uses include setting occupational exposure levels, dose-setting for longer term studies, and classifying mechanism of action. To identify opportunities for regulatory uses of non-animal replacements for acute systemic toxicity tests, we reviewed acute systemic toxicity testing requirements for Brazil, China, Canada, Japan, the European Union, South Korea, and the United States, which participate in the International Cooperation on Alternative Test Methods (ICATM). Our chemical sectors of interest for each jurisdiction were cosmetics and personal care products, consumer chemicals, industrial chemicals, medical devices, and pesticides. We found acute systemic toxicity data were most often required for hazard identification rather than risk assessment. Where animal methods were required, animal reduction methods were typically recommended. However, for many jurisdictions and chemical sectors, non-animal alternatives were not accepted. The most frequently accepted non-animal approaches were test waivers. For example, guidance on medical device testing from the International Standards Organization and for pharmaceuticals from the International Conference on Harmonization both indicate that acute toxicity information can potentially be obtained from other studies. This understanding of international regulatory requirements for acute systemic toxicity testing will inform the development of ICATM’s strategy for the development, acceptance, and implementation of non-animal alternatives to assess the health hazards and risks associated with acute toxicity. This project was funded by the Physicians Committee for Responsible Medicine and with federal funds from the NIH, NIH under Contract No. HHSN273201500010C.

2342 Feasibility of Long-Term Social Housing in Male ICR (CD1) Mice When Providing a Complex Environment


Now globally accepted, social housing has been implemented in most experimental animal species; however, male mice are often housed individually due to aggressive behavior. We previously conducted a 4-week study on social housing of male mice, and the results suggested that social housing of male mice is feasible if animals are provided with an appropriately enriched housing environment. In this study, we further investigated the feasibility of social housing in male mice for a 13-week period. We also investigated the effects of social housing on common endpoints in general toxicity studies and the stress level of the animals by comparing the data to those from single-housed mice. Six-week-old male ICR (CD1) mice were housed for 13 weeks. Seven cages each were set to house single (S, n = 7), pair (P, n = 14), and trio (T, n = 21) groups. Nesting materials and a Mouse Igloo® (Bio-Serv) were supplied to each cage, as previously reported. Tap water (5 mL/kg) was administered orally once daily to mimic the handling usually used in general toxicity studies. Clinical signs observation, body weight and food consumption measurement, clinical pathology assessment (urinalysis, hematology, plasma chemistry), and pathological examinations (necropsy, organ weights, histopathology) were conducted. Plasma corticosterone concentration was analyzed at Week 12 of dosing. During the 13-week period, 1 pair of mice was separated at Week 1 because aggressive behavior and trauma were frequently observed in one of these mice. Six out of the 21 mice were housed socially for 13 weeks. The body weight in the trio housing group was significantly higher from Week 2 than that in the single housing group. The mean absolute bilateral adrenal weights in the social housing groups (P: 4.42 mg, T: 4.16 mg) were comparable to that in the single housing group (4.87 mg). The corticosterone concentrations (means/SD) in the social housing groups (P: 2.52±0.2729, T: 1.899±2.340) tend to be lower than those in the single housing group (14.28±14.05), which included 2 males with markedly high values (32.86 and 30.68 ng/mL). There were no noticeable changes in clinical pathology or histopathology among the groups. In conclusion, social housing in male mice is feasible for this study for up to 13 weeks when providing an appropriately enriched housing environment, with the animals appearing to have lower stress levels than the single-housed animals.

2343 Design Features and Elemental Analysis of the Atomizers in Pod Electronic Cigarettes


The atomizers of electronic cigarettes (ECs) contain metals that transfer to the aerosol upon heating and may present health hazards. This study aimed to analyze 4th-generation EC pod atomizer design features and characterize their elemental/metal composition. Eleven EC pods from six brands/manufacuturers were purchased at local shops and online. Pods were dissected and detailed elemental, and infiltration data were obtained using a Nikon DSLR camera. Elemental analysis and mapping of atomizer components were done using a scanning electron microscope coupled with an energy dispersive x-ray spectroscope. EC Pods varied in size and...
design. The internal atomizer components were similar across brands except for variations occurring mainly in some products’ wicks and filaments. The filaments were either Elnivar (nickel, iron, and chromium) (36.4%), nichrome (36.4%), iron-chromium (18.2%), or nickel (9%). Thick wires present in 55% of the atomizers were mainly nickel and were joined to filaments by brazing. Wire-connecter joints were Elnivar. Metal air tubes were made of Elnivar (50%), nickel, zinc, copper, and tin (37.5%), and nickel and copper (12.5%). Most wicks were silica, while two pods (PHX and Mico) had ceramic wicks with additional minor elements. Connectors contained gold-plated nickel, iron-chromium, and multiple alloys of nickel, zinc, gold, iron, and copper. Wick chambers were made of Elnivar. Outer casings were either nickel, copper-tin, or nickel-copper alloys. Magnets were nickel with minor iron, copper, and sulfur. The atomizers of pods are similar to previous generations with the introduction of ceramic wicks and magnets in the newer generations. Since elements identified in EC components may transfer into aerosols, their health effects and fate in the environment should be further investigated.

2344 Estimation of Screening No-Significant-Risk-Levels (NSRLs) and Product Exposure to β-myrcene and Pulegone

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β-myrcene and pulegone are naturally occurring constituents of plants, and their derived juices and essential oils with wide use as fragrances in cosmetics, soaps, and household products. These chemicals can readily leach into water, under the State of California’s Proposition 65, which requires companies to provide a warning if exposure to the chemical is above a No-Significant-Risk-Level (NSRL). An NSRL is a risk level which represents a lifetime exposure in the general population resulting in one excess case of cancer in a population of 100,000. California’s Office of Environmental Health Hazard Assessment (OEHHAA) has not currently derived NSRLs for either chemical. Lastly, these chemicals are considered only a concern under Proposition 65 if they are of synthetic origin, with products containing β-myrcene and pulegone from naturally occurring sources being excluded from exposure considerations. Using available data from the National Toxicology Program (NTP) data on both chemicals, Exponent ran a quantitative risk assessment, drawing on regulatory and scientific considerations, to derive screening-level NSRLs for both β-myrcene and pulegone. Further, a screening exposure assessment was generated based on common use patterns and expected exposure to cosmetic and soap products. Both chemicals are currently listed for cancer and developmental toxicity in products (from synthetic sources) that would be below the derived screening-level NSRLs. These screening-level NSRLs as well as maximum product concentrations can be used to inform on Proposition 65 compliance determination and risk.

2345 Predicting Oral Acute Toxicity Using the GHS Additivity Equation

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The United Nations Globally Harmonized System for Classification and Labeling (GHS) provides a mathematical approach to estimate the acute toxicity of a mixture based on the combined toxicities of the individual components of the mixture. The authors evaluated how well toxicity values calculated using the GHS formula and the corresponding U.S. Environmental Protection Agency (EPA) and GHS hazard categories agreed with those obtained from in vivo acute toxicity studies of the same formulations. Data were compiled for approximately 700 agrochemical and antimicrobial product formulations, most of which were classified in the less hazardous EPA Categories III and IV and GHS Categories 4, 5, and Not Classified (NC). Although over all concordance was 54% using the EPA classification system and 72% using the GHS system, the majority of discordant results were associated with substances with measured LD₅₀ between 2000 and 5000 mg/kg that were predicted using the additivity equation as having minimal toxicity (i.e., EPA Category IV or GHS NC). Such underclassifications may be of lesser practical concern from a risk assessment perspective than underclassification of more toxic substances. However, for the few toxic substances included in the data set, the additivity equation often predicted a less toxic outcome than the in vivo test. Given the inherent variability of the animal test, it is certainly possible that similar underclassifications could also be observed if the animal test were repeated. We also subdivided the dataset based on formulation type (i.e., antimicrobial cleaning products [AMCP] and agrochemicals) and found that classification concordance was much greater for AMCP (84% and 98%) than for agrochemicals (52% and 70%). These data indicate that acute systemic toxicity of many formulations is not well represented by the sum of their ingredients’ toxicities. This could be because the formulations’ components may interact to result in higher toxicity than is predicted by the GHS equation or because the variability of the animal study is not adequately accounted for in the additivity calculation. This project was funded with federal funds from the NIEHS, NIH under Contract No. HHSN272201300010C. The views expressed above do not necessarily represent the official positions of any federal agency.

2346 Comprehensive Evaluation of Aerosol Constituents in JUUL System Virginia Tobacco 5.0% Using Non-targeted Analysis: Workflow Overview


FDA’s Premarket Tobacco Application (PTA) guidance for Electronic Nicotine Delivery Systems (ENDS) recommends that applicants provide a “list of uniquely identified constituents or chemicals, including -- other toxic chemicals contained within the product or delivered by the product.” In order to provide a comprehensive list of aerosol constituents, quantitative harmful and potentially harmful constituents (PHHCs) analysis was conducted and a semi-quantitative non-targeted analysis (NTA) approach was implemented. This work focuses on the semi-quantitative NTA approach. JUUL System aerosol was generated using a non-intense and intense puffing regimen and the aerosol extract was analyzed using GC-MS and LC-HRMS techniques. All detected compounds were categorized into five groups (ingredients, PHHCs, extractables & leachables, reaction products, and not rationalized/unknown) and the average concentration of each group was estimated. The potential sources and chemical reactions were proposed for each compound to better understand the aerosol composition. Tentative identifications for structures with potential toxicological concern were further evaluated for confirmation of identity. The NTA identified a total of 97 constituents in aerosol from JUUL System Virginia Tobacco 5.0% (VT5%). The constituents identified in the NTA accounted for approximately 0.2% of the total aerosol mass. By comparison, using a chemical structure is a complete list of more than 5000 chemicals. Of the 97 constituents identified in JUUL VT5% by NTA, 35 of these constituents were also identified in cigarette smoke and 62 compounds were unique to JUUL VT5% aerosol. At the low levels present in VT5% aerosol, the constituents identified in the NTA are unlikely to raise toxicological concerns under the conditions and duration of use compared to cigarette smoke. Taken together, the NTA is a valuable analytical tool for a more comprehensive evaluation of aerosols generated from ENDS products.

2347 Regulatory Status of Pesticide Residues in Cannabis: Implications to Medical Use in Neurological Diseases

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All 50 states and Washington DC now have legal access to medical cannabis, recreational cannabis, and/or cannabidiol oil. Despite being considered by some as a “wonder drug”, cannabis is not regulated in the United States as a pharmaceutical. Pesticide contamination has led to public concerns of cannabis safety and multiple recalls by cannabis producers. We analyzed the regulatory documents of 34 medical cannabis programs and compared the pesticide testing requirements and action levels. Six states imposed the strictest US EPA tolerances (i.e. maximum residue levels) for food commodities on up to 400 pesticidal active ingredients (As) in cannabis, while 21 states and Washington DC developed their own action levels. 21 As, including amitriptyline, bifenazate, etozazole, and imidacloprid were regulated in 26 states, while 67 As were regulated in only one state. Dimethomorph showed the largest variation in the action levels, ranging from 0.1 ppm to 60 ppm in 5 states. Discrepancies were also observed in qualifying conditions for medical use. Most states (31) listed movement disorders as qualifying conditions, which include epilepsy, multiple sclerosis, Parkinson’s disease, and seizure. Since seizure is also a symptom of acute pesticide poisoning, we evaluated 33 organophosphates, 10 N-methyl carbamate, and 21 pyrethroid pesticides as well as parquat and diquat herbicides for their potential connections to seizure using Comparative Toxicogenomics Database (CTD). Twenty pesticides and 60 genes were associated with seizure in 597 computationally generated pesticide-gene-phenotype-disease tetramer constructs in CTD, including parquat (199 tetramers), chlorpyrifos (179), and diazinon (60). Superoxide dismutase 2 had the highest number of pesticide interactions (8 pesticides, 59 tetramers), followed by interleukin 18 (7 pesticides, 54 tetramers) and interleukin 6 (8 pesticides, 43 tetramers). Since medical cannabis is a potential route of harm-
2348 Preventing Pneumoconiosis: Review of a Safe Level for Lifetime Occupational Inhalation Exposure to Mica

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Mica is a chemically inert phyllosilicate mineral with a wide range of applications. It naturally occurs as brittle translucent sheets, most of which are processed into ground-up flakes of varying sizes. The industrialization of mining processes—especially crushing, milling, screening, and bagging of mica scrap—led to significant worker exposures and associated health effects into the first half of the twentieth century. Therefore, the American Conference of Governmental Industrial Hygienists (ACGIH) included mica dust in its first publication of Maximum Allowable Concentrations for workers in 1946. Later redefined as Threshold Limit Value-Time-Weighted Average (TLV-TWA), the respirable particulate fraction of mica in air remained essentially unchanged since 1951. However, more recently, the ACGIH 2020 Notice of Intended Changes proposed to reduce the mica TLV-TWA from 3 mg/m³ respirable fraction to 0.1 mg/m³. This review assesses the effectiveness of the ACGIH TLV-TWA for worker protection. Historical records of pneumoconiosis rates in the U.S. workforce were evaluated with respect to the long latency period for disease manifestation. The existing ACGIH TLV-TWA for mica was found to be scientifically justified and protective of workers’ health. Moreover, reducing disease manifestation. The existing ACGIH TLV-TWA for mica was found to be scientifically justified and protective of workers’ health. Moreover, reducing the TLV-TWA was not found to be warranted based on historical data regarding disease associated with occupational exposure to mica dust.

2349 Global Analysis of Human Exposure Pathways and Adverse Effects of Mercury from Skin-Lightening Cosmetics: A Systematic Review

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Mercury (Hg) is a global pollutant that poses a threat to overall human health. Inorganic Hg is a common ingredient found in skin-lightening cosmetics that inhibits melanin production and lightens skin tone. Usage of skin-lightening cosmetics is practiced worldwide, most commonly in African, Asian and Caribbean nations. The skin-lightening cosmetic industry is expected to reach US 31.2 billion by 2024. The Minamata Convention on Mercury has established a limit of 1ppm of Hg in skin-lightening cosmetics, however many products have greatly exceeded this limit. Despite strict bans and regulations, Hg-added skin-lightening cosmetics are still easy to illegally obtain. The main objective of this research was to (1) quantify the amount of Hg human populations worldwide are being exposed to through the use of Hg-added skin-lightening cosmetics; and (2) summarize the adverse health effects associated with such exposures. This was achieved via a systematic search of peer-reviewed scientific literature in four databases (PubMed, Web of Science Core Collection, Scopus, and Toxline) in July of 2018. The initial search identified 1,711 unique scientific articles, of which 25 were ultimately deemed eligible for inclusion after iterative screens at the title, abstract, and whole text levels. A second search was performed in November of 2020 using the same methods, from which another 7 recently published articles were included. All papers were organized according to six sub-domains (“Product Origin,” “Cosmetic Hg Concentrations”, “Usage”, “Biomarkers”, “Health Outcomes”, and “Regulatory Implications”) prior to data extraction and synthesis. In the final extracted dataset, we recorded 371 data entries for cosmetic Hg concentrations, 871 data entries for human biomarkers, 70 data entries for usage practices, and 634 individuals with health related outcomes. This data represents a wide range of Hg exposure through cosmetics; for example, the recorded product concentrations ranged from non-detectable to 45,622 ppm. We are currently analyzing the extracted data to generate higher-level insights on the prevalence of Hg-added skin-lightening cosmetics and their impacts on human health. The findings from this research will be shared with the Secretariat of the Minamata Convention on Mercury and the World Health Organization to further inform decisions on the international regulation of skin-lightening cosmetics.

2350 Retrospective Review on In Vitro Phototoxicity Data Generated in 3D Skin Models to Support the Development of New OECD Test Guideline

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Phototoxicity (also photo-irritation), is a light-induced skin reaction that occurs when photoreactive chemicals are produced to activate effects. Phototoxicity testing of chemicals is mostly conducted in vitro, following the methods described in the OECD test guideline (OECD TG 432), using the Balbc/C 3T3 mouse fibroblast cell line. The test is highly sensitive, but low specificity has been reported in some studies (1). The test has also limitations with testing poorly soluble compounds. The use of 3D reconstructed human epidermis (RHE) tissue models has been proposed as the second tier in an integrated testing strategy to assess potential phototoxic activity, especially for topical exposures (e.g., chemicals, pesticides, cosmetics) (2). We collected published data on phototoxicity testing using various RHE models and protocols. The dataset contains more than 80 materials and over 800 entries utilizing five different RHE models. The analysis conducted on the datasets revealed, that despite some differences between the protocols (e.g., exposure times, dosing, solvents), the RHE have potential not only to distinguish between phototoxic and non-phototoxic materials but also the potential to predict phototoxic potency as demonstrated in limited studies conducted in parallel to clinical studies in human volunteers. This database provides a valuable resource towards achieving regulatory acceptability of this method. This database was funded in part with federal funds from the National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN273201500010C. References: 1. Lynch AM, Wilcox P. Review of the performance of the 3T3 NRU in vitro phototoxicity assay in the pharmaceutical industry. Exp Toxicol Pathol. 2011;63(3):209-14. 2. Liebsch et al. Development of a new in vitro test for dermal phototoxicity using a model of reconstituted human epidermis. ALTEX. 1997;14(4):165-174.

2351 Supporting Efficiencies in Nonclinical Toxicology Studies through Common Templates

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BioCelerate is an industry consortium driving initiatives to increase efficiency in early-stage R&D. Our ongoing projects resulted in the release of a Nonclinical Common Protocol Template (NCPT) for repeat-dose toxicology studies in November 2019, and through efforts to evolve synergistic processes, a companion Nonclinical Common Report Template (NCRT) in November 2020. Benefits to using these common templates for sponsors and CROs include (1) productivity (time/effort/resources) optimization in drafting these documents for each study, (2) improved overall study quality by decreasing errors due to unfamiliarity with protocol/report layout, (3) simpler management of multiple studies, and (4) freeing up time to spend on critical data analysis and review. Importantly, in preparation for the future digitized state of information exchange in toxicologic research, widespread adoption of these common templates will provide a consistent substrate with which to transition these documents into electronic format. For both common templates, streamlined layout and process instructions were defined where large variations in preferences existed. For template development, public feedback from stakeholders was incorporated as part of the template creation process. BioCelerate team collaborated with pharmaco- tox reviewers from the FDA and subsequently hosted an open webinar to gather input from stakeholders. Both templates contain: (1) sections with common text that may be used with little to no editing; (2) major headings that are recommended to not be deleted; however, additional subheadings.
A shift is underway in toxicology. U.S. regulators now confirm the need to integrate modern approaches that better predict human outcomes than the traditionally used animal studies. In late 2019, the Environmental Protection Agency (EPA) Administrator formally committed to replacing mammalian studies by 2035, and within a year the EPA produced a work plan to lead agency activities in achieving this goal. Animal tests are ingrafted in FDA policy and industry practice for all sectors that FDA regulates. With such inertia in place, a similar commitment from the Food and Drug Administration (FDA) Commissioner would help the agency more quickly actualize its goal of integrating improved approaches by redirecting the scientific community. The FDA’s 2017 Predictive Toxicology Roadmap lays a foundation for change in toxicology at the agency. In support of such initiatives, and with the goal of increasing the human relevance of nonclinical drug testing, a growing group of professionals from federal agencies, the private sector and patient, health and research organizations collaborate under the National Institute on Patient Safety Initiative (NIPSI). Through a Drug Discovery Today publication, NIPSI outlined factors that impede integration of new approaches and provide recommendations for addressing these factors. Ongoing projects focus on changing policy, supporting human-based science, and offering industry and regulator training. One policy project involves changing FDA regulations from requiring “animal” data to “nonclinical,” which encompasses animal in vivo, in vitro, and in silico approaches. Another project aimed to establish an evaluation pathway for regulatory acceptance of human biology-based nonclinical approaches at FDA, which the agency launched in late 2020 via the Innovative Science and Technology Approaches for New Drugs (ISTAND) pilot program.

### 2352 Policy Initiatives to Support Use and Development of Human Biology-Based Nonclinical Approaches for Drug Development

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A shift is underway in toxicology. U.S. regulators now confirm the need to integrate modern approaches that better predict human outcomes than the traditionally used animal studies. In late 2019, the Environmental Protection Agency (EPA) Administrator formally committed to replacing mammalian studies by 2035, and within a year the EPA produced a work plan to lead agency activities in achieving this goal. Animal tests are ingrafted in FDA policy and industry practice for all sectors that FDA regulates. With such inertia in place, a similar commitment from the Food and Drug Administration (FDA) Commissioner would help the agency more quickly actualize its goal of integrating improved approaches by redirecting the scientific community. The FDA’s 2017 Predictive Toxicology Roadmap lays a foundation for change in toxicology at the agency. In support of such initiatives, and with the goal of increasing the human relevance of nonclinical drug testing, a growing group of professionals from federal agencies, the private sector and patient, health and research organizations collaborate under the National Institute on Patient Safety Initiative (NIPSI). Through a Drug Discovery Today publication, NIPSI outlined factors that impede integration of new approaches and provide recommendations for addressing these factors. Ongoing projects focus on changing policy, supporting human-based science, and offering industry and regulator training. One policy project involves changing FDA regulations from requiring “animal” data to “nonclinical,” which encompasses animal in vivo, in vitro, and in silico approaches. Another project aimed to establish an evaluation pathway for regulatory acceptance of human biology-based nonclinical approaches at FDA, which the agency launched in late 2020 via the Innovative Science and Technology Approaches for New Drugs (ISTAND) pilot program.

### 2353 Evaluating the Therapeutic Effectiveness of Antidiabetic Drugs to Reduce Tobacco Smoke-Promoted Cerebrovascular Damage after Traumatic Brain Injury

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Traumatic Brain Injury (TBI) is among the most prevalent causes of cerebrovascular and neurological damage worldwide. Current scientific opinion considers that premorbid conditions such as tobacco smoking (TS) exacerbate post-TBI brain damage and impact recovery due to vascular endothelial dysfunction as a result of TS content of reactive oxygen species (ROS), nicotine, and oxidative stress (OS) stimuli targeting the blood-brain barrier (BBB) endothelium. Additionally, due to chronic pathogenic modulators of BBB impairment between chronic cigarette smoking and hyperglycemia (HG), antidiabetic drugs like metformin (MF) and rosiglitazone (RSG) were shown to reduce BBB damage promoted by the chronic TS exposure. Herein, through an *in vitro* study, we evaluated the therapeutic feasibility and effectiveness of antidiabetic drugs to prevent/reduce TS-promoted cerebrovascular damage after TBI and/or improve post-TBI recovery. For this purpose, experiments were conducted on TS-exposed mouse brain microvascular endothelial cells (mBMMEC-P5) induced by a valid *in vitro* TBI model. Quantitative RT-PCR analysis was applied to assess the expression of Nrf2 (a critical antioxidant transcription factor), NF-κB (a pro-inflammatory transcript factor), inflammation, adhesion molecules (PECAM-1 and VCAM-1) as well as tight junction proteins associated with BBB integrity including, ZO-1, Occludin, and Claudin-5. Additionally, we evaluated other pathogenic parameters including intracellular ROS generation, glutathione, thrombomodulin, and pro-inflammatory cytokines IL-6, IL-10, and TNF-α. The results revealed that MF and RSG can effectively decrease the harmful effect of TS on intracellular ROS and OS generated in response to TS treatment. RSG and MF also upregulated NRF2 expression and its downstream effector NQO1 and HO-1, decreased TSimduced endothelial inflammation and loss of barrier integrity, and restored the thrombomodulin release from cells which decreased upon TS exposure. In conclusion, through the underlying linking mechanism, antidiabetic drugs could be repurposed to reduce BBB damage and subsequent severity of TBI outcomes caused by TS dysregulation of the cellular antioxidant response system.
**2356** Assessing Contractility of 3D IPSC-Derived Muscle Models for Safety and Discovery at High-Throughput Using a Novel Label-Free Magnetic Detection Method

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Skeletal muscle developed from induced pluripotent stem cells (IPSCs) is being used more frequently in drug development. However, current methods for assessing drug-induced effects on contractility use 2D cell models, which lack the complexity of 3D muscle tissue. We have designed a novel system that can leverage the complexity of 3D cellular models in a high-throughput format necessary for contemporary drug discovery.

**2357** Irinotecan Decreases Intestinal UDP-Glucuronosyltransferase (UGT)1A1 via TLRs/MyD88 Pathway: A Novel Mechanism of Chemotherapy-Induced Diarrhea

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Irinotecan is a first-line treatment for many gastrointestinal (GI) cancers. However, its fatal GI toxicity raises safety concern. Irinotecan is the prodrug of 7-Ethyl-10-hydoxy-camptothecin (SN-38). In liver, irinotecan generates deactivated metabolite SN-38G via hepatic UDP-glucuronosyltransferase (UGT)1A1. Consequently, SN-38G is excreted by GI tract as the result of activation by microbial β-glucuronidase (GUS) to yield reactive metabolite, SN-38. In the present study, we treated C57BL6/J mice with 50 mg/kg irinotecan via intraperitoneal (i.p.) injection once daily for two days. Mice were sacrificed at 4, 12h, 24h, and 48h after the first dose. Following this, wildtype (C57BL6/J), TLR2-/- (B6.129-Tlr2tm1Jbr), TLR4-/- (B6.129-Tlr4tm1Jbr), and MyD88-/- (B6.129zPgk-Mylpp1B1.1 Defr) mice were given 50 mg/kg irinotecan via i.p. injection once daily for two days. Intestine and liver were collected for RT-qPCR. Previous studies showed that mice started diarrhea after 6 days consecutively after injection of irinotecan. Our results in wildtype mice showed that Ugt1a1 expression in GI tract decreased after 24h since first dose before showing any diarrhea. PXR and CAR, the two main nuclear receptors governing intestinal Ugt1a1 via a TLRs/MyD88-dependent mechanism, which eventually triggers the onset of diarrhea. Our finding unveils a novel mechanism underlying irinotecan-induced GI toxicity. It provides a new direction to prevent chemotherapy side effect.

**2358** In Vivo Imaging and Evaluation of Drug-Drug Interaction Risk Arising via Hepatobiliary Transporters

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Clinically relevant drug-drug interactions (DDIs) may lead to potentiated or reduced efficacy, and/or may increase or reduce toxicity to liver or other tissues. DDIs may arise via inhibition of hepatic transporters that mediate drug uptake and/or biliary excretion. Although transporter interactions can be assessed using in vitro assays, these do not enable accurate prediction of effects that occur in vivo. We have investigated the use of dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) as a novel in vivo method for investigation of transporter mediated DDIs. DCE-MRI enables in vivo visualisation of hepatic transporter mediated uptake and efflux of the contrast agent gadoxetate. When analysed using a compartmental kinetic model of gadoxetate disposition, gadoxetate DCE-MRI data provide quantitative rate constants for hepatic gadoxetate uptake (ku) and biliary excretion (kb). These processes are mediated primarily by Organic Anion Transport Polypeptides (OATPs) and Multidrug Resistance Protein Type 2 (MRP2), respectively. The effects of test drugs that inhibit rat oatp, and human OATP, on hepatic gadoxetate ku and kb was evaluated in adult male Wistar rats (approx. 250g body weight). Single drug doses were given via intravenous (iv) infusions, then after a 20 min interval gadoxetate (25 μmol/kg) was administered iv and DCE-MRI images were acquired for 58 sec intervals at 30 time points. Drug doses were selected, via pharmacokinetic modelling and simulation, to achieve rat peripheral blood plasma concentrations equivalent to steady-state human blood plasma concentrations. The pharmacokinetic simulations predict that the UGT2B1-mediated deactivation of gadoxetate exposure in vivo, whereas the other tested drugs (asunaprevir, bosentan, ketoconazole and pioglitazone) did not. Gadoxetate ku values were determined 20 min after iv administration of dose vehicle and then, in the same animals and after a minimum 48 hr washout interval, following drug administration (n=6 per group). Gadoxetate ku was reduced (p < 0.01) after administration of rifampicin at 2 mg/kg (mean±SD, dose: 0.92±0.17; vehicle: 1.00±0.24), or cyclosporin at 25 mg/kg (mean±SD, dose: 0.08±0.02; vehicle: 0.10±0.24), but not after dosing of asunaprevir at 5 mg/kg, bosentan at 2 mg/kg, ketoconazole at 3 mg/kg or pioglitazone at 0.4 mg/kg. These results indicate that gadoxetate DCE-MRI may aid assessment of hepatic transporter-mediated DDI risk.
I_{ac} is mostly responsible in other cell types; (5) drug-induced action potential (AP) alternans with beat-to-beat variations occur at high pacing rates in P cells. These results suggested that quantitative profiling of transmural and rate-dependent properties can be essential to evaluate drug-induced arrhythmogenic risks, and may provide mechanistic insights into drug-induced arrhythmias.

2361 Repurposing Small Drug Molecules for Tissue Regeneration: Vascular Development
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Muscloskeletal injuries are common and represent a significant economic burden and may result in long-term health issue that require surgical intervention to promote tissue regeneration. Currently, tissue regeneration is severely hindered by lack of oxygen, nutrient support, and waste removal due to suboptimal vascular regrowth. The gold standard vascular endothelial growth factor (VEGF) approach has shown to be inefficient due to its 30 minute half-life. Drug molecules such as norepinephrine (NE), phenylephrine, vancomycin, and nicotine have been shown to increase angiogenesis via activation of VEGF pathways. Upregulation of VEGF pathways is notably an associated adverse effect rather than a therapeutic effect. To our knowledge, these drugs have not been thoroughly studied outside of cancer models. Their involvement in angiogenesis at controlled low doses may provide new avenues for tissue engineering and wound healing applications. Therefore, the purpose of this study is to explore NE as an alternative to VEGF in scaffolding formulations to promote angiogenesis in tissue engineering. Rats were implanted with NE loaded scaffolds for 4 weeks subcutaneously. Vascularization was evaluated by histology of implanted scaffolds using hematoxylin and eosin staining as well as immunohistochemistry for CD31. Histological analysis of stained sections showed prominent vasculature development throughout isolated rat tissue as compared to vasculature present only on the peripheral edges in the control scaffold. The results show NE incorporated scaffolds with optimal release profile improve vascularization in implanted scaffolds subcutaneously. This could help address significant challenges in tissue engineering. The signaling mechanism(s) involved is unknown and currently being investigated.

2362 Cardiac Drug Amiodarone Accumulation and Neurotoxicity in the iPSC-Derived Human 3D Model BrainSpheres
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Amiodarone, a broadly used class III antiarrhythmic drug, was linked to side effects such as headache, dizziness, tremor and ataxia, suggesting neurotoxicity. Clinical effects generally require serum concentration >0.5 µM, whereas increased risk of toxicity is associated with a serum concentration >3µM. The neurotoxicity and distribution kinetics of amiodarone were evaluated, using the iPSC-derived human 3D model BrainSpheres (B5), comprising neurons, astrocytes and oligodendrocytes, and showing spontaneous electrophysiological activity. BS were exposed to amiodarone for 48h or repeatedly between week 6 and 7 in vitro. Survival was assessed at the end of each exposure scenario and after one week of washout following the full week of exposure. Cytotoxicity was evaluated by MTT assay in BS exposed to concentrations ranging from 0.625 to 20µM of amiodarone. At the end of the repeated exposure, IC50 was found at 2.4µM, and at 2.3µM after the washout period. At the concentration of 7.5µM, RT-qPCR analysis showed strong increase in expression of astrocytic markers after 48h of exposure and of neuronal markers compared to controls. TempOSeq analysis revealed that amiodarone targets several neuronal markers after repeated exposure and of neuronal markers after repeated exposure. This could help address significant challenges in tissue engineering. The signaling mechanism(s) involved is unknown and currently being investigated.

2363 Factors Influencing Prediction of Bromodichloromethane (BDCM) in Exhaled Breath
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Confidence in the predictive capability of a physiologically based pharmacokinetic (PBPK) model is increased when the model is demonstrated to predict multiple pharmacokinetic outcomes under differing exposure conditions. We previously showed that our multi-route human BDCM PBPK model adequately predicts both blood and urine BDCM concentrations from human exposure studies; activities in these studies included drinking, bathing, showering and swimming. Exhaled breath BDCM (exBDCM) was adequately predicted in subjects actively swimming. However, exBDCM was over-predicted by 7-10 fold when compared to median experimentally measured exBDCM for unmoving subjects immersed in pool water in two different studies. We used the BDCM PBPK model to sequentially explore possible physiological explanations for this observation, including temperature-dependence of cardiac output (QC), alveolar ventilation rate (Qp), skin permeability and skin blood flow. These parameters were selected based on results of literature review and sensitivity analysis. Increasing QC and Qp from original resting values to ones more consistent with those estimated for unmoving (non-hypothermic) water-immersed subjects resulted in 4-7 fold over-prediction of exBDCM. Decreasing skin blood flow by 50% resulted in slightly improved predictions, i.e. over-prediction of exBDCM in the range of 3-6 fold. Also, decreasing apparent skin permeability by 75% resulted in over-prediction of exBDCM that was less than 2 fold. Decreased apparent skin permeability with decreasing water temperature has been reported for chloroform, which like BDCM is a trihalomethane. Relative decreases in skin blood flow on the order of 3-fold have been documented in the literature when water temperature is decreased from 40-34 °C. In studies where water temperature has been reported, the typical range for showering is 38-40 °C, whereas pool water is more typically in the range of 26-28 °C. The impact of water immersion on QC and Qp is temperature-dependent and complex. Our results illustrate the utility of a PBPK model for simulation of unique and complex physiology. The views expressed in this abstract are those of the authors and do not necessarily represent the views or the policies of the US Environmental Protection Agency.

2364 Development and Application of a Physiologically Based Pharmacokinetic Model to Predict Oxytetracycline Tissue Distribution and Withdrawal Intervals in Market-Age Sheep
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Oxytetracycline (OTC) is a widely used antibiotic in food-producing animals. Extralabel use of OTC may lead to violative residues in edible tissues. To ensure human food safety, it is important to have a quantitative tool to predict scientifically-based withdrawal intervals (WDIs) of OTC in food animals. This study focuses on developing a physiologically based pharmacokinetic (PBPK) model for predicting plasma and tissue concentrations of OTC in sheep and goats. The PBPK model included seven compartments: plasma, lung, liver, kidney, muscle, fat, and rest of the body. The model was calibrated with independent data sets from the Food Animal Residue Avoidance Databank (FARRAD). Results of application of this model with independent data sets from FARRAD and USDA. The model was evaluated with independent data sets in the literature. Results of application of this model with independent data sets in the literature. Results of application of this model with independent data sets in the literature. Results of application of this model with independent data sets in the literature. Results of application of this model with independent data sets in the literature. Results of application of this model with independent data sets in the literature. Results of application of this model with independent data sets in the literature. Results of application of this model with independent data sets in the literature. Results of application of this model with independent data sets in the literature. Results of application of this model with independent data sets in the literature. Results of application of this model with independent data sets in the literature.
It is important to have mechanism-based quantitative models to simulate and extrapolate the biodistribution of nanoparticle-based new drug formulations within and between animals and humans. Further evaluation of a nanomedicine’s delivery efficiency to target sites, their safety and risk needs user-friendly tools but such tools are lacking. In this study, a web-based graphical user interface (GUI) was developed to predict the absorption, biodistribution, and elimination of gold nanoparticles (AuNPs) ranging from 1.4-200 nm in adult rats based on a membrane-limited physiologically based pharmacokinetic (PBPK) model with multiple administration routes including intravenous (IV), oral gavage, intratracheal instillation, and endotracheal inhalation. This PBPK model was rigorously optimized using a Bayesian hierarchical approach, and then converted to a web-based interface with R Shiny. In addition, multivariate linear regression analyses were conducted to gain deeper insights into the relationships between the physicochemical characteristics of AuNPs (e.g., size, surface area, dose, zeta potential, and NP numbers) and PBPK parameters. This new model adequately described the tissue distribution of different types of AuNPs in multiple target organs, including lungs, liver, spleen, and gastrointestinal (GI) tract, despite differences in the kinetic behaviors and physicochemical properties following diverse exposure scenarios. Regression results showed endocytic/phagocytic uptake rate was associated with size or surface area of AuNPs for all administered routes, while zeta potential was an important parameter for exocytic release following IV administration. This new PBPK-based web interface is a useful tool to predict the distribution of numerous types of AuNPs in rats following different routes of administration, and serves as a basis for extrapolating to other NPs and to humans to facilitate delivery efficiency, safety, and risk assessment of nanomedicines.

Quantitative understanding of dermal absorption is needed in the chemical risk assessment of products that are exposed to skin. Although computational methods can address data gaps in the risk assessments, there is currently no single validated prediction method to reliably estimate skin permeation for all exposure scenarios, chemicals, and formulations. This study evaluates a mechanistic dermal absorption model and presents a workflow for in vitro-in vivo extrapolation. The mechanistic dermal absorption model by Dancik et al. (2013, Advanced Drug Delivery Reviews, vol. 65, pp. 221-236) predicts in vitro and in vivo skin penetration of small molecules and has been programmed into MoBi (part of Bayer’s Open Systems Pharmacology Suite) and is available on Github (https://github.com/Open-Systems-Pharmacology/Skin-permeation-model). Hewitt et al. (2019, Journal of Applied Toxicology, vol. 40, pp. 403-415) presents results of in vitro permeation tests (IVPT) performed on 56 chemicals of cosmetic relevance. Predictions of the cumulative dermal absorption ($Q_{cum}$) of these compounds were generated by the MoBi model. The nominal model estimates were mostly within one order of magnitude of IVPT observations (47 of 56 chemicals) and were more likely to overestimate (26 of 56 chemicals) than underestimate (2 of 56 chemicals) dermal absorption by more than 2-fold. A custom R package was written to execute a workflow for optimizing the MoBi model to in vitro data and for subsequent prediction of in vivo dermal absorption. First, a sensitivity analysis identifies the model parameters requiring optimization. Next, a Markov Chain Monte Carlo algorithm derives a joint posterior distribution of the parameters given IVPT data. Finally, the likely range of in vivo dermal absorption is estimated by simulating the MoBi model in vivo using samples from the posterior distribution. The workflow was applied to seven compounds tested in vivo. In each case, the optimized model yielded close agreement between observed and predicted $Q_{cum}$. The predictive performance of the optimized model was variable when compared against in vivo experimental observations. The optimized model for ibuprofen from an unoccluded IVPT closely predicted the in vivo absorption whereas the dose was temporarily occluded. This example demonstrates the potential of the workflow to extrapolate IVPT findings to novel in vivo contexts for risk assessment.

**2365** Development of a Web-Based Interface to Facilitate Nanomedicine Design and Safety Assessment through the Prediction of Biodistribution of Nanoparticles

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Community exposures to ethylene oxide (ETO) have recently made headlines due to increased concerns about the carcinogenicity of this widely used substance for medical equipment sterilization. ETO has been assessed by measuring the hemoglobin adduct of ETO (N-(2-hydroxyethyl) valine (HEV)) in blood. ETO is formed endogenously in the body from ethylene (Et) normal physiological processes and from exogenous Et exposure via occupational and environmental sources. Therefore, HEV reflects the cumulative exogenous and endogenous exposures to Et and ETO. In this study, we adapted the human inhalation PBPK model published by Filszer and Klein by including age, gender and cigarette smoking components. The improved model was used to predict HEV levels using inhalation exposure scenarios reported in the literature. Predictions using this model were compared with measured HEV in the U.S. general population. The performances of the model were evaluated on biological rationale, reliability and range of application. As reported in the National Health and Nutritional Examination Survey (2015-2016), the HEV concentration for smokers (186 pmol/g globin) was seven times greater than non-smokers (27 pmol/g globin). The PBPK model estimated that for the U.S. non-smokers, 2.37 pmol/g globin (8.78%) corresponds to exogenous exposure to Et and ETO, and 24.63 pmol/g globin (91.2 %) corresponds to the endogenous sources. For U.S smokers’ population, 85.7% of HEV (159.4 pmol/g globin) is estimated to be associated with cigarette smoking, 1.3% (2.4 pmol/g globin) with exogeneous non-smoking exposure, and 13% (24.2 pmol/g globin) with endogenous sources. HEV level increases with the number of cigarettes smoked per day with a rate of 8.06 pmol HEV/g globin/cigarette. The adapted model may provide a new quantitative assessment tool to help inform the risk assessment of Et/ETO in general populations. 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.
Recent studies have assessed the efficacy of the intranasal route (INR) of dosing for hormones and drugs that can be rapidly absorbed into the brain and act via interactions with endocrine or neuro receptors in specific brain regions. Examples include drugs like estradiol and testosterone that act systemically in part through receptor-based signaling in the pituitary gland and hypothalamus at the base of the cerebrum, in close proximity to nasal sinus tissues. The nasal mucosa has appreciable, highly vascularized surface area and contains low levels of metabolizing enzymes relative to those in the GI tract or liver; this route thus permits direct access of the drug to various brain regions and circumspects first-pass metabolism. A number of animal studies have demonstrated that hormones and drugs administered by INR can enter the brain but only a small number of these have examined brain and endocrine target tissue concentrations. A pharmacokinetic model containing target tissues with endocrine receptors would aid in understanding the timing versus concentration relationships resulting from INR dosing of hormones/dugs that act on these receptors. To account for tissue distribution relevant to endocrine events, existing physiological-based pharmacokinetic (PBPK) models for endocrine active compounds was modified to include 1) INR dosing, and 2) compartments for the hypothalamic-pituitary-gonadal (HPG) axis, which included the whole brain, hypothalamus, pituitary, adrenals, thyroid, and testes/ovaries. All compartments were described as flow-limited; tissue and blood flow parameters were taken from the literature. To keep the model generally applicable, no metabolism was included in the endocrine tissues and no pharmacodynamic components were included. With INR dosing, a portion of the administered dose will be transported directly from the nasal cavity to the brain while some portion will enter systemic circulation; this was accounted for by brain distribution data for other drugs, which can reasonably be used as a surrogate for several endocrine active compounds, administered from both the INR and intravenous routes in rodents. This model provides a general framework for estimating target tissue concentrations of endocrine active compounds in HPG axis tissues resulting from INR dosing and can be modified by adjusting the tissue: blood partition coefficients for specific compounds and by including known information on metabolic pathways.

Drug-induced ventricular arrhythmia, e.g. life-threatening torsade de points, has been a major concern since the early stage of drug development. In vitro human ether-a-go-go related gene (hERG) inhibition assay, or more recently multi-channel inhibition assay, has been effective to predict drug-induced cardio toxicity. However, experiments at sub-cellular or cellular level could be insufficient in predicting tissue-level drug-induced arrhythmogenic events and providing mechanistic insights underlying these events. In addition, it remains unclear if intrinsic tissue-level electrophysiological or structural heterogeneities in the Purkinje-Ventricular system (PVS) have a role in the onset and development of drug-induced ventricular arrhythmogenicity. Here, a group of one-dimensional (1D) heterogeneous PVS tissue models with intrinsic electrophysiologic or structural variations were developed to quantitatively predict and investigate drug-induced arrhythmogenicity at physiological pacing frequencies (1–4Hz) and effective free therapeutic plasma concentrations (EFTPC). We found that: (1) electrophysiological heterogeneities across Purkinje-Ventricular junctions (PVJ), the abundance and localization of mid-myocardial tissue can have a substantial impact on the onset and development of drug-induced arrhythmogenic events; (2) An increase in the abundance of mid-myocardial tissue can enhance drug-induced arrhythmogenicity with the application of quinidine or bepridil, and induce abnormal excitation patterns such as electric alternans of excitation at high pacing frequencies with a larger conduction delay across the PVJ; (3) with the application of mexiteline, ranolazine or verapamil, only minor drug-induced QT prolongation can be observed, likely due to concurrent blockade of both inward (I\textsubscript{Na}), or outward (I\textsubscript{K1}) ionic currents; (6) little drug-induced arrhythmogenicity can be observed with the application of sotalol, chlorpromazine or ondansetron. Our results suggested that heterogeneous tissue models of the PVS may serve as an invaluable tool to quantitatively predict drug-induced arrhythmogenic events, and provide mechanistic insights into drug-induced ventricular arrhythmias.
NCNonalcoholic fatty liver disease (NAFLD) impacts close to 25-30% of the US population. Hepatic steatosis is a biological manifestation of NAFLD and is defined by an increase of more than 5% of lipid content in the liver. The complex biological mechanisms that disrupt lipid homeostasis leading to lipid retention in hepatocytes are ultimately due to dysregulation of four cellular functions: hepatic fatty acid (FA) uptake, de novo FA and lipid synthesis, FA oxidation, and lipid efflux. Exogenous factors that can cause disruption of these processes include lifestyle factors, diet and exposure to many environmental chemicals such as carbon tetrachloride (CCL4). Experimental in vivo research has shown that CCL4 decreased the levels of hepatic very low-density lipoprotein (VLDL), a major carrier of lipids from liver to general blood circulation. This mechanism was included in a quantitative systems toxicology (QST) model describing CCL4 impact on hepatic steatosis. The QST model consisted of a physiologically based pharmacokinetic (PBPK) model for CCL4 and a mechanistic model describing the cascading biological events leading to excessive retention of free fatty acids and triglycerides in the liver. The overall model was calibrated using in vivo data from rats orally exposed to CCL4. The calibrated model will serve as a quantitative method to establish dose-response relationships for CCL4 and hepatic steatosis. When human CCL4 PBPK models are employed, the calibrated QST can be quantitively extrapolated to address health risk assessment of the chemical in inducing hepatic steatosis in humans. This abstract does not necessarily reflect US EPA policy.

During pregnancy certain diseases require medicines to reduce the risk of harm to mother and fetus. One such example is labetalol commonly prescribed for gestational hypertension. Since many physiological changes occur in pregnancy which can affect pharmacokinetics (PK) alternative or adaptive dosing regimens might be necessary to ensure efficacious and safe drug therapy. However, it is can be difficult to accurately dose pregnant women based on data as they are often excluded from clinical trials due to ethical concerns. Physiologically based pharmacokinetic (PBPK) modeling is a series of mathematical equations that can predict drug PK for dynamically changing life-stages, like pregnancy. Labetalol is metabolized by UGT2B7 and UGT1A1 which are not extensively characterized in pregnancy. The goal of this work is to quantify parameter contributions most influential in describing pregnancy PK and evaluate model uncertainties in data sparse life-stages using PBPK modeling. The pregnancy PBPK model for labetalol was constructed de novo and was evaluated using clinical PK data. Trimester-specific parameter sensitivities and their respective pregnancy related physiological changes were used to estimate individual parameter contributions to the cumulative change in AUC. For a highly lipophilic and protein bound compound, change in body weight (BW) and albumin (Alb) contributed greatly to the overall changes in AUC across trimesters (BW: 25%, 42%, 37%; Alb: 56%, 41%, 29%, respectively). Although UGT1A1 ontogeny was characterized with the limited available data, the activity change of the main metabolizing enzyme, UGT2B7, during pregnancy was not described a priori due to lack of data. The pregnancy PBPK model lacking this component still captured 56.7% of total change in AUC for trimester 1 and 88.5% in trimester 3. Based on the model analysis the potential contribution of UGT2B7 activity change was estimated. The described method allows for the estimation of the extent each parameter contributes to pregnancy related PK changes. This could help prioritize parameterization of future PBPK models and build model confidence in data-sparse life-stages, thus improving prediction capabilities and sensitivity assessments of PBPK modeling for use as a regulatory tool.

Synthesis of 11 steroid hormones in human adrenocortical carcinoma cells (H295R) was measured in a high-throughput steroidogenesis assay (HT-295R) for ~2000 chemicals in single concentration (sc) and 653 chemicals in multi-concentration (mc) screening as part of ToxCast. The mc data were previously modeled with a Mahalanobis distance-based approach to indicate effect size, and positive or negative results, for this 11 hormone system. However, data are unavailable for many chemicals of interest. This work addresses the hypothesis that physicochemical and/or chemical structural features may provide useful indication of HT-H295R bioactivity. Chemicals without effect in sc screening were combined with chemicals screened in mc, which were largely positive, for a training set of 1380 chemicals. ChemoType enrichment analysis revealed sub-structural features that were over-represented with respect to bioactivity. A global random forest model using 13 predicted physicochemical features, such as logP and vapor pressure, resulted in a balanced accuracy (BA) of 74%, which was not substantially improved by addition of ToxPrints. In contrast, a nearest neighbor approach using 2 different chemical fingerprints (ToxPrints, Morgan) and a Jaccard similarity, performed better. The best nearest neighbor models demonstrated BAs of 84-85%, using 1% of nearest neighbors and stringent probabilities for positive (>0.8) and negative (<0.1) outcomes among neighbors. These models used 228 and 243 chemicals based on ToxPrints or Morgan fingerprints, respectively, to define neighborhood. Only 7700 of a 10000 chemical inventory of interest had structural descriptors; removing chemicals with HT-H295R data yielded a test set of 6302 chemicals. Of these, ~4400 were labeled as equivocal. Approximately 1100-1800 chemicals were predicted negative, and 86-143 were predicted positive, depending on the descriptors used. Twelve chemicals were positive and 643 chemicals were negative for both descriptor sets. Using a nearest neighbor approach to structure-activity prediction identified substructures for which existing bioactivity data on related substances may help inform gaps in screening data to support the Endocrine Disruptor Screening Program and any further in vitro or in silico evaluation. This abstract does not necessarily reflect US EPA policy.

Investigation of normal human thyroid function using in vitro culture systems is dependent on cells that recapitulate physiology of differentiated thyrocytes. Primary thyrocytes retain features of the native organ but have limited lifespan in culture. Immortalized thyrocytes offer an alternative if challenges with phenotypic stability can be overcome to retain functional features of primary cells. CI-SCREEN immortalization technology was applied to normal human thyroid tissue. Four human thyroid epithelial cell lines (hThyEC) lines were generated and characterized for transgene integration, biomarker expression, genomic stability, and proliferation rates. Thyroid Stimulating Hormone (TSH)-dependent morphology, thyroglobulin production, thyroxine hormone synthesis, and viability were assessed using conventional 2D monolayer and 3D microtissue culture formats in hThyEC or hThyEC medium. Despite differential transgene profiles, the lines had similar biomarker expression patterns and proliferation rates. In 2D culture, there was no thyroxine synthesis or changes in viability, but TSH-dependent thyroglobulin production was more significant for several lines in hThyEC than hThyEC medium. Comparatively, in 3D microtissues, TSH-dependent thyroglobulin induction was greater for cell lines in hThyEC medium. Synthesis of thyroxine in one cell line was higher than background with TSH exposure, but not significantly different than control. In summary, select human thyroid cell lines exhibit morphological and functional features of primary thyrocytes and can be a novel resource for in vitro disease modeling and toxicity testing. The views expressed are those of the authors and do not necessarily reflect the views or policies of the US Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.
Organophosphate esters (OPEs), used extensively as flame retardants and plasticizers, are found ubiquitously in the environment. However, little information is available on their safety. Previous studies suggested that exposure to OPEs may be detrimental to female fertility in humans. The goal of this study was to elucidate the effects of OPEs on the phenotype and function of KGN human granulosa cells. We hypothesized that OPEs alter the function of the cells to a greater extent than the enzymatic activities of the primary metabolites of the aromatic compounds they are derived from. Further, only DINP affected cells to a greater extent than MEHP.

In summary, the effects of plasticizers on phenotypic parameters were cell-line specific. Further, only DINP affected cells to a greater extent than MEHP.

In silico analysis of the effects of bisphenol analogs on human adrenocarcinoma cells (H295R cells) revealed that exposure to a single OPE may alter the histology of adrenal glands and disrupt steroidogenesis. However, we are constantly exposed to a broad array of OPEs. One of the major sources of exposure is through the ingestion and inhalation of dust. We hypothesize that exposure to the environmentally relevant mixture of 13 OPEs found in Canadian house dust will affect human adrenal cell function. To test this hypothesis, H295R human adrenal cells were exposed to vehicle (DMSO) or to dilutions of 1/1,000,000 X to 1/3000 X of the house dust OPE mixture for 48 h, where 1 X is equivalent to the OPE concentrations found in 5.005g of Canadian house dust. High content screening was used to assess the effects on cell survival, oxidative stress levels, lysosomes, autophagic activity and lipid droplets. OPE exposure to dilutions of 1/3,000 and 1/10,000 were cytotoxic, i.e., greater than 30% cell death, and thus were not used for further analysis. Cellular characteristics, such as oxidative stress levels, numbers of lysosomes and mitochondrial activity, were not affected by OPE exposure. However, total lipid droplet area per cell increased significantly due to an increase in the number of lipid droplets per cell, starting from 1/1,000,000 X. Thus, this environmentally-relevant OPE mixture altered lipid droplet dynamics in adrenal cells. Supported by Canadian Institutes of Health Research and McGill University.

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Bisphenol A (BPA; 4,4’-(1-methylpentylidene)bispbenol) has been used for the manufacture of polycarbonate plastics and epoxy resins. BPA and its analogs (bispbenols; BPs) are widely detected in the environment, and their potential estrogenic effects on wild animals are of great concern. It is known that some of BPs elicit estrogen-like responses. However, little information is available for the effect of BPs on the estrogen receptor (ER) signaling pathway in birds. There are also still unanswered questions about the structural preference of BPs by ER and the molecular mechanism of ER activation. To assess the transcriptional potency of BPs via chicken ERα (cERα), we measured the agonistic and antagonistic activities of 26 bispbenol analogs (BPs) by using the in vitro reporter gene assay system that cERα was transiently expressed in COS-1 cells. Results showed that 4,4’-methylenebispbenol (4,4’-BP), 2,2’-methylenebispbenol (2,2’-BP), bis(4-hydroxyphenyl)methane (HPB), and 4,4’-thiobispbenol (TBP) had agonistic activities, whereas BPA, 4,4’-2,2’-trifluoro-1-(trifluoromethyl)bispbenol (BFAP), 4,4’-ethylenedibispbenol (BEPB), 2,2’-methylenebispbenol (2,2’-BP), 4,4’-sulfonylbispbenol (BSBP), 4,4’-(2,2-dichloroethylenyl)bispbenol (BPC2), 4,4’-(1-phenylbispbenol) (BPB), and 1,1’-(1-methylpentylidene)bispbenol (DPP) had no transcriptional activity. Co-treatment with 17β-estradiol had no transcriptional activity. These results suggested that this approach may serve to identify chemicals most likely to be responsible replacements. Supported by CIHR, McGill University and the Macau Government.

Together, these data suggest that this approach can aid in the screening and selection of responsible replacements for further testing and analysis. Supported by CIHR and McGill University.
Development of Battery of In Vitro Assays for High-Throughput Identification of Thyroid Hormone Disrupting Chemicals

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Thyroid Hormone Disrupting Chemicals (TDCs) are xenobiotics that can interfere thyroid hormone (TH) system. This disruption of TH signaling in human is suspected to play a role in induction of developmental problems such as low IQ scores, cognitive and behavioral defects in children, and thyroid autoimmune disorders, thyroid neoplasms, subclinical and clinical hypothyroidism and increased cardiovascular risk due to altered lipid metabolism in adults. In this project, we are proposing and optimizing a battery of in vitro assays for screening of chemicals for their thyroid disrupting properties. The design is based on adverse outcome pathway concept (AOP) addressing priority molecular initiating events (MIEs) in the thyroid hormone regulation, which covers MIEs in TH synthesis, transport, signaling and metabolism. Thyroid peroxidase (TPO) inhibition luminometric assay was performed to evaluate the interaction of TDCs with the activity of TPO enzyme that has a strong relation to the TH synthesis. Moreover, TPO from human and rat cells is used for interspecies comparison. Thyroxine-transhydroxyn (T4-TTR) binding assay was performed to evaluate effects of TDCs on TH transport. Interactions of TDCs with TH receptor and Aryl hydrocarbon receptor, which is linked with TH metabolism, were assessed using human cell lines stably transfected with reporter gene under control of the respective receptor activity. All the assays are performed in 96 well plate format to provide for high throughput screening capacity. We characterized the battery performance on a set of 20 prioritized human exposure relevant model chemicals including environmental pollutants, natural compounds and pharmaceuticals. Several of the chemicals activated TH receptor but none of them induced AhR-mediated activity. However, bisphenol A, propylthiouracil and benzophenone had a strong impact in TPO assay, and the results correlated very well between human and rat models. Binding inhibition was shown for many of the chemicals in TTR assay. Thus, the effectiveness of high-throughput assays in the battery was shown and after further validation they could serve for hazard assessment of human exposure relevant chemicals. The project has received funding from the EU H2020 research and innovation programme project EROG (grant agreement No.825753).

Endocrine-Disrupting Chemicals Increase Early Testosterone Surge in Male Rodents


Male rodents and primates experience a transient activation of the hypothalamic-pituitary-gonadal (HPG) axis that triggers a surge of serum testosterone (T) at birth known as mini-puberty. Reproductive research has suggested postnatal T production can act as an in vivo screening tool for endocrine disrupting chemicals (EDC). This T surge has been shown to be sensitive to mixtures of EDCs, resulting in enhanced male reproductive disease, yet, little is known about extension to the central nervous system. Complicating the use of T secretion as an in vivo screen for developmental exposure to EDCs, the T surge in male mice is reported to be a transient, short increase immediately following birth. Here, we quantified the T surge hourly following birth in control C57Bl/6 mice. Secondly, we utilized multiple EDC mixtures to disrupt the T surge and compared their effects on central nervous system development. Our data show that in C57BL/6J male mice, the T surge peaks two hours post-birth and serum T levels return to baseline 6 hours post-birth. As expected, there was no surge in T in female mice. Secondly, we used a complex EDC mixture, a reduced binary mixture, and a mixture of polychlorinated biphenyls (PCBs: Aroclor 1221) to compare EDC effects on T surge at birth and lifelong T production. To address the possibility that the T surge is sensitive to multiple EDC mixtures, C57BL/6J mouse dams were exposed to Aroclor 1221 (1mg/kg), a complex EDC mixture (Atrazine (ATR: 10mg/kg), Perfluorooctanoic acid (PFOA: 0.1mg/kg), Bisphenol-A (BPA: 50µg/kg), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD: 0.036µg/kg)) or a reduced binary BPA+PFOA mixture (BPA: 50µg/kg and PFOA: 1mg/kg) orally administered via treats from gestational day 0 until birth. All three mixtures significantly elevated serum T at birth in male mice. Additionally, all three exposures lowered T concentrations in adulthood and disrupted adult social behavior: increased isolation preference and reduced social communication. In conclusion, these data indicate that multiple EDC mixtures and individual EDCs alter mini-puberty, despite having different direct molecular modes of action along the HPG axis. Mini-puberty may provide a sensitive endocrine window underlying vulnerability for male-dependent neurotoxicity. These data also highlight the importance of timing in the determination of EDC action, as both early androgenic and later anti-androgenic changes were found. Supported by P30 ES001247.

Mechanistic Insights into the Toxicity of Mammary Toxicants Using the BCScreen Gene Panel and the TempO-seq Targeted RNA Sequencing Platform

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Over 200 chemicals have been associated with altered mammary gland development and breast cancer, yet the molecular mechanisms by which these diverse chemicals affect the breast remains poorly understood. We compared gene expression profiles of eight suspected or recognized mammary toxicants in MCF-7 cells at four doses (1 nM to 10 µM) using the TempO-seq platform and the Breast Carcinogen Screen (BCScreen) gene panel, which represents biological processes relevant to breast carcinogenesis and mammary gland development. The known weak estrogen genistein (Gen), bisphenol A (BPA), and butylbenzyl phthalate (BBP) had similar gene expression profiles as estradiol-treated cells and these profiles were enriched for cell cycle and genotoxicity related genes, particularly at higher doses. Notably, perfluorooctanoic acid (PFOA) and 1,4-benzoquinone (BQ) expression changes were also enriched for genes associated with cell cycle and genotoxicity processes, respectively, with the opposite direction of effect as the weak estrogens. PFAS and BQ also had more differentially expressed genes at the lowest dose compared to higher doses. PFOA expression profiles were enriched for targets of the E2F transcriptional factor family, E2F1 and E2F4, which control key elements of mammary gland development. Disregulation of the E2F regulatory pathway may be an important modulator of chemical-induced mammary toxicity. Targeted transcriptomics identified biological processes and transcriptional regulators that may be involved in altering mammary gland development or carcinogenesis in response to mammary toxicants. This approach has the potential for being utilized in chemical screening and prioritization to identify other chemicals as potential mammary toxicants.

Reducing Uncertainties in Quantitative Thyroid AOPs by Analysis of Thyroid Hormones in the Neonatal Rat Brain


Adequate supplies of thyroid hormones (TH) are essential for brain development. Serum TH is routinely used in regulatory toxicology to identify chemicals with the potential to induce neurological deficits. Thus, serum TH occupancy a pivotal position in adverse outcome pathways (AOP) for developmental neurotoxicity. Recent evidence, however, suggests that downstream neurological deficits do not always accompany reductions in serum TH. As such, TH levels in the fetal and neonatal brain may be necessary for the development of quantitative AOPs of thyroid-dependent neurotoxicity, yet technological challenges have limited their report. We optimized protocols for brain sample preparation to reduce matrix interference, enhance recoveries, and assess TH levels in neonatal rat brain to address this need. Male and female offspring from Long-Evans rats (n=5) were euthanized on postnatal days (PN) 0, 2, 6, and 14, and whole-brain or neocortex was collected. Isootopically-labeled internal and surrogate standards were added to 100 mg of brain homogenate, and TH were separated from lipids via a modified Folch extraction. Anion exchange solid-phase extraction further removed residual phospholipids. Quantification of TH was achieved by liquid chromatography-mass spectrometry using scheduled multiple reaction monitoring (sMRM) with a method detection limit of 0.005 ng/g for each TH. The mean recovery for all TH ranged from 70% to 125%. Linearity (r²=0.998) was observed from 0.005 ng/g to 25.6 ng/g for each TH. Based on surrogate standards, intra- and inter-day precisions were determined to be ± 15%. Brain TH concentrations increased over developmental time, T4 and T3 ranging from 0.560 to 3.05 ng/g and 0.900 to 5.00 ng/g, respectively, from PN0 to PN14. Only minor TH concentration differences were observed in perfused versus nonperfused samples, and no sex-dependent differences were evident in brain or serum TH concentrations at these ages. Incorporating brain TH measures in studies of developmental exposure to thyroid disrupting chemicals is feasible; improving potential neurotoxicity predictions will advance the utility of AOPs for risk determination. This work does not reflect US EPA policy.
2390 Endocrine-Disrupting Effects of Polychlorinated Biphenyl (PCB153) on Human Placental Cells

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Polychlorinated biphenyls (PCBs) are persistent environmental organic pollutants and considered as endocrine disruptors. Despite their worldwide ban, PCBs can still be detected in the environment and general population due to their high chemical stability and lipophilic properties. PCBs can cross the placental barrier and accumulate in the placenta. Several studies have described PCB-dependent adverse effects on human fetal growth, including increased risk for intrauterine growth restrictions, changes in endocrine function and hormone metabolism, and neurological deficits. Estrogens affect a variety of tissues, including reproductive tissues, bones, and brain. Estrogen is mediated by the two receptors isomers: estrogen receptor alpha (ERα) and beta (ERβ). Estrogen receptor alpha (ERα) is the major regulator of placental cell function. PCBs can promote cell proliferation and cell survival in both normal and transformed epithelial cells by modifying the expression of hormone responsive genes involved in the cell cycle, cell growth and/or programmed cell death. The aim of this study was to examine the estrogenic activity of PCB congener 153 in human placental trophoblast (BeWo) cells. Cells were treated with various concentrations of PCB153, or estrogen alone or in combination for different exposure time. Cell proliferation, viability and estrogen transcription factor activation were assessed. Concentration (0.01-10µM) of estrogen significantly induced cell viability and proliferation in a concentration- and time-dependent manner. Exposure of BeWo cells to low concentration (0.05µM) PCB 153 significantly reduced estrogen-induced cell viability. The ERα antagonist, Fulvestrant, and the ERβ antagonist, PHTPP, blocked the effects of PCB153, indicating that this effect is mediated through an ER-dependent pathway involving the control of cell growth and proliferation in the placenta. PCB153 significantly antagonized estrogen-dependent transcription by estrogen. This indicates that PCB153 can compete with ER for binding to the ER transcription factor. Our data suggest that maternal contamination with PCBs could disrupt placental endocrine activity, which in turn, could lead to adverse effects on pregnancy outcomes. In addition, these results demonstrate that the current environmental exposure levels of PCBs are a risk to reproductive health. Supported by Title III.

2391 Implementation of an In Vitro FTC-238-hrTPO-Based Tyrosine Oxidation Method to Evaluate Chemicals for Thyroperoxidase (TPO)-Inhibiting Properties


Within the EU-NETVAL network of European validation laboratories currently various in vitro methods are developed and validated targeting different modes of action (MoA) of thyroid disruption. One of the targeted MoAs is inhibition of thyroperoxidase (TPO), an enzyme critical in thyroid hormone synthesis. Although various methods for investigating inhibition of TPO are described in literature, most methods are not completely animal-free and as such there is a need for alternative methods. In the current work an in vitro method to assess chemicals for potential inhibition of TPO mediated iodination using FTC-238-hrTPO cell homogenates [1] was developed and implemented to be ready for validation. The model substrate L-tyrosine was incubated with potassium iodide, FTC-238-hrTPO cell homogenate and peroxide. TPO enzymatically converts L-tyrosine into the metabolite mono-iodotyrosine (MIT), which was quantified using UPLC-MS/MS as a direct measurement of TPO activity. After optimizing the method by modifying pH, time of reaction, and substrate concentration, the model inhibitors (evaluated as potential positive control and reference items) methimazole, propylthiouracil, genistein and sulfamethazine were tested. This resulted in IC50 values of 0.6 µM, 2.2 µM, 0.7 µM and 13.9 µM, respectively. The non-inhibitor bis(2-ethylhexyl)phthalate (used as negative control item) showed no inhibition as expected. These outcomes demonstrate that the developed method is a promising tool for medium-throughput screening of chemicals on inhibition of TPO, hereby contributing to the setup of a robust toolbox of relevant in vitro methods to predict thyroid disruption in the framework of the ECHA/EFSA Guidance on identification of endocrine disrupting chemicals (chemicals that interfere with the normal hormonal function and disrupt the endocrine system). This guidance was kindly provided by Professor Josef Köhle, Charité, Berlin, Germany. [2] Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009; EFSA Journal 06/2018.

2392 The Androgenic Impact of AKR1C3 and the 11-Oxidized Androgenic Ovary Syndrome (PCOS)


Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women of reproductive age. The androgen excess (AE) of PCOS is believed to promote cardio-metabolic diseases. Our goal is to identify targets for endocrine disrupting chemicals (EDCs) that could influence this syndrome. AE correlates with insulin resistance (IR) in these women and may be the driving factor for lipotoxicity. Aldo-keto reductase family 1 member C3 (AKR1C3) is induced by inflammatory signals and may be central in driving the AE of PCOS. AKR1C3 predominantly catalyzes conversion of peripheral androgens such Δ4-androsten-3,17-dione (4AD) to testosterone (T) and 5α-androstan-3,17-dione (5AD) to 5α-dihydrotestosterone (DHT). T and DHT are potent agonists for the androgen receptor (AR), which upregulate de novo lipogenesis. Fatty acid synthase (FASN) is known to be induced by androgens and may be responsible for this altered lipid profile in mature adipocytes. AKR1C3 overexpression in adipocytes, in response to insulin, could increase androgen receptor signaling and increase de novo lipogenesis. This lipid overflow could exacerbate insulin resistance through a feedforward mechanism and promote the lipotoxic profile seen in PCOS. Additionally, the adrenal 11-oxgenated androgens may be an alternative source of AE and have been reported as the dominant androgens of PCOS. We hypothesize that insulin induction of AKR1C3 and the conversion of 11-oxygenated androis into 11K-T and 11K-DHT may be influenced by EDCs in adipocytes to potentiate hyperandrogenism and lipotoxicity. Using differentiated LX2/Samp1/Gale-Behm Syndrome (GSB) adipocytes as a model, we have shown that insulin increased AKR1C3 expression by >600 fold at the RNA level. Using pharmacological inhibition, we determined that insulin induction of AKR1C3 utilizes the PI3K/AKT/mTOR pathway as measured by RT-qPCR and immunoblot assay. Using a R-PHLC discontinuous assay and post-reaction derivatization with dinitrophenylhydrazine, we determined the Km and Km for the conversion of 11-ketoandrosten-3,17-dione (11K-4AD) to 11-ketotestosterone (11K-T) and 11-ketoandrostren-3,17-dione (11K-5AD) to 11-ketohydroxysterosterone (11K-DHT) using recombinant AKR1C3. We find that 11K-AD and 11K-5AD are superior substrates for AKR1C3 when compared to 4AD and 5AD. This research identifies AKR1C3, AR, and FASN as targets for EDCs that may affect hyperandrogenism and AR signaling in PCOS and the ensuing lipotoxicity.

2393 Multi-generational Metabolomics of Bisphenol A Exposure in C57BL/6J Mice: Sexually Dimorphic Exposure Effects and Connections with the Circadian Clock

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Endocrine disrupting chemicals (EDCs) disrupt hormone action and are linked to development of metabolic disease. Bisphenol A (BPA) is a high proportion volume chemical used in manufacture of polycarbonate plastics and epoxy resins. BPA is persistent in the environment and biomonitoring studies reveal pervasive human exposure. Rodent models demonstrate BPA-induced impacts on body weight, pancreatic function, glucose homeostasis and insulin signaling. However, specific molecular mechanisms of BPA-induced diabetes and obesity related outcomes remain to be elucidated. In parallel, the circadian clock is a critical regulator of metabolic homeostasis and widespread crosstalk exists between circadian and endocrine systems, the potential for EDCs to disrupt circadian-driven cellular metabolism or physiology is not well characterized. The goal of this research is to understand how BPA exposure may contribute to the development of metabolic disease through disruptions in energy handling. A mass spectrometry-based metabolomics workflow was utilized to assess biochemical impacts induced by BPA in a multi-generational maternal exposure model in which pregnant dams were subject to dietary BPA exposure at 10 µg/kg/day (lower dose), 10 mg/kg/day (upper dose) or control (7% corn oil). 340 aqueous phase metabolites were profiled from liver and adipose tissue (adult) and adipose tissue (adult) harvested from C57BL/6J mice. Multivariate modeling using orthogonal partial least squares discriminant analysis (OPLS-DA) revealed generation, gender and dose-specific metabolic fingerprints of BPA exposure in liver and adipose tissues, and an acute metabolic signature of gestational BPA exposure in male and female neonate liver. Across datasets, BPA perturbed a range of biochemical pathways including amino acid metabolism, purine and pyrimidine metabolism, and redox and energy metabolism-related pathways, and altered abundance of metabolites previously characterized as circadian in C57BL/6J mouse liver. BPA-induced biochemical pathway impacts warrant further investigation as these path-
for the differentiation of PC CD11b+ populations which was not prominent in BM. TCDD also influenced T-cell maturation causing thymic atrophy. Thus, when thymocytes were cultured with TCDD in the presence or absence of RSV, TUNEL assay showed RSV significantly decreased TCDD-induced apoptosis of thymocytes. Overall, the data demonstrated that RSV decreased TCDD-induced immune suppression by altering the dynamics of various cell populations in terms of numbers, metabolism and immunosuppressive potency. Supported by NIH grants P01AT003961, P20GM103641, R01AI23947, R01AI29798 and R01ES19133.

**2397 Systematic Map of the Immune Effects of Hexavalent Chromium (Cr(VI))**

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Cr(VI) is a naturally occurring heavy metal that poses cancer and non-cancer health hazards to several target organ systems, including the hepatobiary, reproductive, and immune systems. Cr(VI) is a well-studied chemical, and the databases of human and animal studies evaluating these effects are comprehensive and challenging to assess efficiently. Evidence mapping is a useful tool for identifying and organizing the literature to facilitate further analysis and to identify data gaps and research needs. Here, we applied evidence mapping principles to organize the human and animal databases for effects of exposure to Cr(VI) on the immune system. A systematic literature search strategy was developed to identify and document reports on immune effects of Cr(VI). Literature was subsequently screened for relevance using Population, Exposures, Comparators, and Outcomes (PECO) criteria. We identified 11 human and 23 animal papers that evaluated PECO-relevant immune effects. An additional 53 supplemental papers including human, animal and in vitro studies reporting immune-relevant mechanistic data were also identified. Relevant experimental design and study outcome information were extracted and organized into a literature inventory. The endpoints evaluated in each study were categorized according to the type of outcome reported in terms of the immune system. There were 8 papers that investigated the most informative outcomes for immune system hazard determination: antibody responses, host resistance, hypersensitivity, ex vivo white blood cell function, immune organ pathology, immunoglobulin levels, immune organ weights, and white blood cell counts and differentials. The outcomes most commonly studied were contact hypersensitivity, immune organ weights, and white blood cell counts and differentials. These effects were important for nutrient cycling and energy homeostasis and have a strong aspect of circadian regulation. Supported by NIEHS P30ES013508 and T32ES019851, NGMS K12GM081259 and NIDDK R01DK115932.

**2395 Resveratrol Inhibits TCDD-Mediated Induction of Myeloid-Derived Suppressor Cells and Their Functions**

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Previously, we showed that 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), an AhR ligand and a potent and persistent toxicant, induced murine myeloid-derived suppressor cells (MDSC) by migration from bone marrow (BM) to peritoneal cavity (PC). In the current study, we show that Resveratrol (3,4,5-trihydroxy-trans-stilbene; RSV), yet another AhR ligand, reduces TCDD-induced MDSC induction. The number of Cd11b+Gr1+ cells induced by TCDD in the PC hydroxy-trans-stilbene; RSV), yet another AhR ligand, reduces TCDD-induced MDSC induction. The number of Cd11b+Gr1+ cells induced by TCDD in the PC through the body, sit at the hub of numerous physiological processes and diseases. We have demonstrated that CPC potently inhibits function of RBL-2H3 mast cells, including their ability to degranulate, which is the release of bioactive substances including histamine. Degranulation inhibition occurred at non-cytotoxic CPC doses as low as 1 micromol, ~1000-fold lower than the concentrations found in consumer products. We have investigated the molecular mechanisms underlying the inhibition of mast cell degranulation by CPC. We have shown that CPC inhibits store-operated calcium entry (SOCE), a core mediator of the degranulation pathway. Using the genetically encoded voltage indicator ArcLight A242 and confocal microscopy, we have shown that CPC does not interfere with key contributors of SOCE, plasma membrane potential and cytosolic pH, in mast cells. The negatively charged plasma membrane lipid phosphatidylinositol 4,5 bisphosphate (PIP₂) is also a key player in SOCE. CPC displaces the PIP₂-binding protein, MARCKS, from PIP₂, suggesting PIP₂-mediated mechanism for CPC inhibition of SOCE. This research provides biochemical mechanisms underlying the effects of CPC on immune signaling and allow prediction of CPC effects on cell disparate cell types that share similar signaling elements.

**2394 Reproducibility of Adipogenic Chemical Responses in the 3T3-L1 Pre-Adipocyte Model System: An Interlaboratory Study**

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The 3T3-L1 murine preadipocyte line is an established cell culture model for screening metabolic disrupting chemicals (MDCs). Despite a need to accurately identify MDCs for further evaluation, relatively little research has been performed to comprehensively evaluate reproducibility across laboratories, assess factors that might contribute to varying degrees of differentiation between laboratories, and to standardize protocols. As such, the goals of this study were to assess interlaboratory variability of efficacy and potency outcomes for triglyceride accumulation and preadipocyte proliferation using the 3T3-L1 preadipocyte cell assay to test chemicals. Ten laboratories from five different countries participated. Each lab was asked to evaluate one control chemical (rosiglitazone) and three blinded test chemicals using: 1) their laboratory-specific 3T3-L1 cells and their lab-specific differentiation protocol, 2) 3T3-L1 cells provided by the study with their own laboratory-specific differentiation protocol, 3) laboratory-specific 3T3-L1 cells with a shared differentiation protocol, and 4) 3T3-L1 cells provided by the study with a shared differentiation protocol. Blinded responses were analyzed by the coordinating laboratory. Results suggest that while the magnitude and range of bioactivities reported varied considerably across laboratories and test conditions, the presence or absence of activity for each tested chemical was consistent, which suggests that most laboratories would accurately assess activity or inactivity. Activity determinations were much more consistent for triglyceride accumulation (63-95% agreement) than for preadipocyte proliferation (50-70% agreement). Greater consistency in measurements was most often observed for the shared cell line/shared protocol assessment, suggesting that reproducibility could be improved through harmonization of cell sourcing and differentiation protocols, thus increasing confidence in reported outcomes. As such, working to develop a harmonized adipogenic differentiation protocol may represent the best strategy for improving consistency of adipogenic responses using the 3T3-L1 model across laboratories.

**2396 Antimicrobial Agent Cetpyridinium Chloride Inhibits Immune Cell Function**

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Cetpyridinium chloride (CPC) is a positively charged antimicrobial used in consumer products such as mouthwashes at concentrations up to 3 milli-molar, thus exposing humans to high concentrations. There is minimal information on eukaryotic toxicology of CPC; hence, there is urgent need for information since humans and wildlife are being exposed to CPC. Mast cells, ubiquitous throughout the body, sit at the hub of numerous physiological processes and diseases. We have demonstrated that CPC potently inhibits function of RBL-2H3 mast cells, including their ability to degranulate, which is the release of bioactive substances including histamine. Degranulation inhibition occurred at non-cytotoxic CPC doses as low as 1 micromol, ~1000-fold lower than the concentrations found in consumer products. We have investigated the molecular mechanisms underlying the inhibition of mast cell degranulation by CPC. We have shown that CPC inhibits store-operated calcium entry (SOCE), a core mediator of the degranulation pathway. Using the genetically encoded voltage indicator ArcLight A242 and confocal microscopy, we have shown that CPC does not interfere with key contributors of SOCE, plasma membrane potential and cytosolic pH, in mast cells. The negatively charged plasma membrane lipid phosphatidylinositol 4,5 bisphosphate (PIP₂) is also a key player in SOCE. CPC displaces the PIP₂-binding protein, MARCKS, from PIP₂, suggesting PIP₂-mediated mechanism for CPC inhibition of SOCE. This research provides biochemical mechanisms underlying the effects of CPC on immune signaling and allow prediction of CPC effects on cell disparate cell types that share similar signaling elements.

Virtual 2021 SOT Annual Meeting and ToxExpo
Triclosan is an antimicrobial chemical used in healthcare settings and workers can be exposed to triclosan on the skin. Exposure to triclosan has previously been associated with increased rates of aeroallergy and sensitization. Although not identified as a sensitizer, triclosan augmented the allergic response in a mouse model of asthma. The skin is the largest organ in the body and the skin barrier is critical for protection from environmental and microbial exposures. Disruptions in the skin barrier are associated with allergic diseases in humans and disruptions in the integrity of the skin barrier contribute to increased severity of contact hypersensitivity in mouse models. The purpose of this study was to investigate the impact of triclosan exposure on the skin barrier integrity using an in vitro skin model. Triclosan (0.05-0.2%) or acetone vehicle was applied on the air-exposed surface of reconstructed human epidermis (Epiderm) and tissues were collected for gene expression analysis 24 or 48 hours later. Expression of keratin-10 and keratin-14 was decreased 24 hours post triclosan exposure, but unchanged 48 hours post triclosan exposure. This result suggests that exposure to triclosan altered the expression of skin barrier genes early-on post exposure. To assess permeability of the skin, reconstructed human epidermis was exposed to a small molecule, Lucifer Yellow, for 2 hours following 24 or 48 hours of triclosan exposure. Tissues were collected for fluorescence microscopy and culture media were collected to assess the presence of Lucifer Yellow, suggesting passage of the molecule through the skin. The fluorescence intensity in the culture media was elevated at 48 hours but not 24 hours post triclosan exposure, suggesting that the reconstructed human epidermis had increased permeability to Lucifer Yellow 48 hours post triclosan exposure. Taken together, these results suggest that exposure to triclosan disrupts the normal expression pattern of skin barrier genes and this leads to increased permeability of the skin. Alterations in the skin barrier integrity due to dermal exposure to triclosan may play a role in increasing the risk of developing allergic diseases.

Triclosan is currently in clinical trials to assess its potential to alleviate influenza infection, and CPC is currently in clinical trials to assess its potential to alleviate influenza infection. Here we present super-resolution microscopy data indicating that certain cannabinoids, including CBD, possess immune modulatory properties.

The COVID-19 pandemic raises significance for a potential influenza therapeutically, cetylpyridinium chloride (CPC), a positively-charged quaternary ammonium antibacterial agent. Recent studies indicate that CPC may alleviate influenza infection, and CPC is currently in clinical trials to assess its effects on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) morbidity. However, there is a dearth of information regarding the mechanism of how CPC might affect virus-host interaction or immune cell function. Previous studies showed the importance of clustering of influenza viral protein hemagglutinin (HA) and interaction of HA with negatively-charged membrane phospholipids, in influenza infection. Here we present super-resolution microscopy data indicating that CPC (at non-cytotoxic doses, 5-10 µM) reduces HA density and number of HA molecules per cluster within the NIH-3T3 fibroblast plasma membrane, while also destabilizing clusters of PIP2, which is also a key player in immune cell signaling. Furthermore, we have found that CPC interferes with membrane localization of three different PIP2-binding proteins: along with HA, myristoylated alanine-rich C-kinase substrate (MARCKS) and Pleckstrin homology domain in both mammalian fibroblasts and mast cells. This disruption of PIP2 is correlated with inhibition of mast cell function, beginning at 1 µM CPC. Nanoscale co-localization of HA with PIP2 in the plasma membrane, is drastically reduced by CPC, offering a mechanism underlying CPC disruption of influenza. Acquired results inform safe CPC dosage levels in OTC products but also potential pharmacological use of this drug as an influenza therapeutically to reduce global deaths from viral disease.
**2402** The Trichloroethylene Metabolite S-(1,2-dichlorovinyl)-L-cysteine Suppresses Inflammatory Pathways and Cytokine Release in a Macrophage Cell Model

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Trichloroethylene (TCE) is a prevalent prevalent groundwater contaminant found in hundreds of Superfund sites. Previous studies showed that TCE suppresses the capacity of the lungs of mice to fight off pathogenic infections. While pregnancy is a critical period that is sensitive to TCE exposure, little is known about the immunosuppressive effects of TCE during pregnancy. We previously demonstrated that DCVC, a reactive nephrotoxic nephrotoxic TCE metabolite, suppresses the immune/inflammatory response to pathogens in human fetal membrane explants. However, because fetal membranes contain an array of cell types including fibroblasts, trophoblasts, epithelial cells and resident macrophages, the immunosuppressive effects of DCVC in specific cell types remains unknown. Similar to the molecular mechanisms underpinning these effects are poorly understood. To gain insight into these factors, we used a macrophage cell model (THP-1) to investigate transcriptomic and cytokine responses to DCVC treatment with or without pro-inflammatory stimulation with lipopolysaccharide (LPS). THP-1 cells were differentiated into a macrophage phenotype before undergoing the following treatments: control (no treatment), DCVC (10 μM), LPS (100 ng/mL) or DCVC (10 μM)+LPS (100 ng/mL). We profiled THP-1 cellular transcriptomes via RNA-sequencing and collected culture media for quantification of cytokine release using ELISAs. Principle component analysis of the transcriptomic profiles showed a clear separation between treatment and controls. Moreover, pathway analysis identified multiple inflammatory response pathways downregulated in DCVC+LPS treatments versus LPS-only treatments, including: "responsiveness to cytokine" (FDR=7.1x10^-12), "response to cytokine" (FDR=1x10^-13), and "leukocyte activation" (FDR=1x10^-13). Notably, there were 1,399 genes differentially expressed between the LPS and DCVC+LPS treatments, indicating significant modulation of the transcriptomic response pathways. These observations that each treatment caused a significant number of gene expression changes compared to the control group (FDR<0.05 and log2-fold change magnitude>2.5).

2403 **Alteration in THP-1 Cell Cytokine Production Following In Vitro Cyclophosphamide, Cyclosporin, Dexamethasone, or Tacrolimus Representative Immunosuppressants**


Although many immune suppressive drugs have contributed for the cancer chemotherapy, organ transplant, but immune alterations induced by the immunosuppressive drugs have not been thoroughly investigated. In addition, not much studies have been undertaken on cytokine or chemokine profiling affected by administration of immunosuppressants. This study aims to evaluate the immunosuppressive effects of the selected immunosuppressive drugs induced by co-treatment on THP-1 cell line. The α1-2 transcripts coding for IgA1-2 respectively are not expressed in SKW WT cells. Conversely, the Qt-PCR studies show suppressed pro-inflammatory responses in macrophages, indicating a plausible explanation for at least some of the immunosuppression observed in the fetal membranes. Supported by the following: P42ES017198, UL1TR002240 and P30ES017885.

2404 **Potential Role of AhR in Antibody Production by Human B Cells**

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Aryl hydrocarbon receptor (AhR) mediates the immunosuppressive effects of 2,3,7,8 -tetrachlorodibenzo-p-dioxin (TCDD) in murine B cells. The effects of AhR activation on the regulation of expression of human immunoglobulin isotypes have been little explored. We co-transfected naive B cell lines with a reporter construct to measure expression of the reporter gene driven by AhR. When B cells were treated with 100 nM dexamethasone (Dex), we observed that expression of IgG isotypes was significantly increased compared to Dex alone. Treatment with concomitant administration of 100 nM TCDD resulted in a significant decrease in the expression of IgG isotypes. These results indicate that TCDD significantly inhibits the expression of IgG isotypes, potentially suggesting a role for AhR in the regulation of antibody production.

2405 **Immune and Tumor-Promoting Effects of Inorganic Arsenic Exposure on Murine Macrophages**


Inorganic arsenic (iAs) is a carcinogenic contaminant used for drinking in many parts of the world, including the U.S. The World Health Organization set a 10 µg/L safety limit for arsenic in drinking water, yet as many as 140 million people worldwide may still be exposed at levels above that threshold. Arsenic has a series of adverse health effects, among which is a confirmed IARC carcinogen and also affects the immune system. The purpose of this study is to elucidate the effects of arsenic on macrophages in the context of cancer with in vitro and ex vivo exposure paradigms. Briefly, RAW 264.7 macrophages, or macrophages cultured from bone marrow harvested from 8-12 week old adult C57BL/6 mice, were treated with different doses of iAs (0.0001 - 1 µM) during differentiation, and stimulated with either M0 (M-CSF alone), M1 (LPS and IFN-γ) or M2 (IL-4 and IL-13) polarization ligands. Culture supernatant was analyzed for cytokines and nitric oxide (NO) production. Cytokine analysis revealed differences between iAs-treated and nontreated macrophages that were unique to sex, and stage of stimulation. For example, M0 females showed a dose-dependent increase in CCL3 and CCL4 production, while M1 females exhibited an inverted U-shape response with increasing doses of iAs. Males, however, showed no significant changes in these select analytes. Additionally, exposure to iAs altered macrophage NO production in a sex-dependent manner. Using flow cytometric analysis, we also observed changes in expression of M1 vs. M2 polarization markers, such as CD86 and Arg-1, respectively. These results suggest that iAs skews macrophages to the M2 phenotype. Because of the role of M2 macrophages in cancer, we investigated how iAs-exposed macrophages respond to tumor cell conditioned media. Macrophages exposed to iAs had increased migratory capacity toward cancer cell conditioned media, which may in part be attributed to their increased expression of chemokine receptor CCR2. Further, in a coculture model, cancer cells cultured with iAs-exposed macrophages showed increased proliferation compared to controls. Future mechanistic investigation and pathway analysis will explain how iAs specifically targets macrophage polarization and how iAs-exposed macrophages contribute to tumor progression. This research contributes to our understanding of the full spectrum of adverse health effects.
2406 Characterization of the Early Immune Response to Clozapine in Rats: Insights into the Mechanism of Idiosyncratic Drug-Induced Agranulocytosis

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Clozapine has unparalleled efficacy in the treatment of refractory schizophrenia but is infrequently prescribed due to the serious risk of idiosyncratic drug-induced agranulocytosis (IDIAG). While the mechanism of IDIAG is unknown, evidence suggests that it involves an aberrant adaptive immune response against clozapine-modified proteins. Progression to IDIAG is rare, yet most patients starting clozapine experience an innate immune response with paradoxical neutrophilia and elevated proinflammatory mediators that resolves with continued treatment. Therefore, the goal of this work was to characterize the onset of the innate response to clozapine in rats, with a focus on the involvement of myeloperoxidase (MPO), an enzyme that can bioactivate clozapine) and inflammasome activation (a protein complex that activates caspase 1 and IL-1 cytokines). Female Sprague Dawley rats were administered clozapine (30 mg/kg, i.p.) and monitored up to 24 hours. Blood counts showed increased neutrophils and decreased lymphocytes by 3 hours, with changes resolving by 24 hours. Using flow cytometry, increased B cells was noted at 3 hours, as well as decreased blood and spleen T cell subsets. Increased neutrophils, monocytes, and natural killer cells were also observed in the spleen. This was accompanied by elevated protein levels of proinflammatory mediators, including IL-1β, CXCL1 (a chemotactant), and neutrophil elastase in the plasma, spleen, and bone marrow as early as 1.5 hours. Treatment with fluoperiapine, a structural analogue of clozapine, did not cause neutrophilia or increase CXCL1. Clozapine-induced neutrophilia was moderately dampened by pre-treatment with a caspase 1 inhibitor (VX-765) and several immune changes in the blood and spleen were attenuated by pre-treatment with an MPO inhibitor (PF-1355). Thus, clozapine rapidly induced organ-specific immune changes, some of which may have been attenuated by inhibition of reactive metabolite formation (i.e., MPO inhibition) or inhibition of inflammasome activation (i.e., caspase 1 inhibition). Ultimately, a better mechanistic understanding of the immune response to clozapine may reveal ways to prevent or treat IDIAG, enabling safer use of this highly effective medication in patients. Funded by CIHR and scholarships/awards from University of Toronto, Leslie Dan Faculty of Pharmacy, Ontario Graduate Scholarship Program, and MITACS.

2407 Thyroid Disrupting Chemicals in Mixture Perturb Thyocyte Differentiation in Xenopus laevis

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Water contaminants can include thyroid disrupting chemicals that cause varied health effects in humans and wildlife. Thyroid hormones (TH) are crucial regulators of metabolism rate, growth and differentiation in all vertebrates. The perinatal stage of development is most reliant on TH and is most vulnerable to perturbations by thyroid disrupting chemicals. Notably, dysregulation of TH signaling during perinatal development can weaken T cell function in maturity, raising the question of whether thyroid disrupting chemicals can perturb thyocyte development acutely. Using Xenopus laevis tadpoles as an experimental organism, we determined thyroid disrupting effects and thyocyte alterations following short-term exposure to a mixture of naphthalene, ethylene glycol, ethoxylated nonylphenol and octylphenol at concentrations similar to those found in contaminated water. Besides hypertrophy-like pathology in the thyroid gland and delayed metamorphosis, exposure to the mixture antagonized TRβ-induced transcription of the kruppel-like factor 9 (Klf9) transcription factor and significantly raised thyroid stimulating hormone gene expression in the brain, both genes known to modulate thyocyte differentiation. Importantly, exposure to this mixture reduced the number of immature cortical and mature CD8+ thyocytes, while co-exposure with exogenous thyroid hormones abolished the effect. When each chemical of the mixture was individually tested, only ethylene glycol could induce significant antagonist effects on brain and intra-thymic gene expression, but reduction in CD8+ thyocyte numbers could not be rescued by co-exposure to exogenous thyroid hormones. These results suggest that chemicals together in mixture are more potent to perturb thyocyte development. Progression through the thyroid hormone pathway than each chemical alone.

2408 Phenotypic Stability and Application of a Self-Replicating Murine Alveolar Macrophage Model Derived from Fetal Liver


Occupational exposure to crystalline silica (cSiO2) is associated with multiple airway diseases and etiologically linked to the development of autoimmune disease. Elucidating cSiO2’s effects on alveolar macrophages (AM), a primary line of defense against inhaled particles, is crucial for understanding how cSiO2 elicits toxicity. A major obstacle to in vitro mechanistic studies of AMs is the paucity of AMs attainable from a single mouse (~15E6). The goal of this research was to characterize the phenotypic stability of Max Planck Institute (MPI) cells, a self-replicating AM surrogate, and to investigate how these cells respond to cSiO2 in vitro. MPI cells were obtained by culturing fetal liver monocytes with the cytokine GM-CSF, a growth factor critical to AM maturation. We compared early MPI cells (<1 mo in culture) to freshly isolated AMs and found that both are Siglec Fhi, CD11chi and CD14lo and express high mRNA levels of Siglec f, Itgax (encodes CD11c), Marco, and Pparγ and low levels of CD14. However, when cells were cultured >1 mo (late MPI cells), surface and/or gene expression of Siglec F, CD11c, MARCO, and PPARy decreased and CD14 increased. Concurrently, the cells underwent a morphological shift from a round morphology akin to AMs, to more spindle-shaped cells. We next assessed the functional implications of these phenotypic changes on the ability of the cells to phagocytose cSiO2. Early MPI cells more effectively phagocytosed cSiO2 (administered at 50 and 100 µg/cm2) compared to late MPI cells, as seen by light microscopy. cSiO2 engulfment preceded cell death, with most cSiO2 cleared from the cells within the first 3 h, and a substantial increase in propidium iodide (PI)-positive dead cells observed between 4 and 5 h. Late MPI cells, which did not phagocytose as many cSiO2 particles, exhibited less cell death (~50% PI-positive at 5 h, compared to ~80% PI-positive in early MPI cells). Lastly, we assessed extracellular concentrations of the cytokines IL-1α and IL-1β, which are known to be released by AMs following exposure to cSiO2. Both early and late MPI cells released IL-1 cytokines, with significantly greater concentrations detected for the early MPI cells (>5-fold). The higher levels of IL-1 cytokines observed in early MPI cells could not be explained by increased lytic cell death alone, suggesting higher expression of these cytokines in early MPI cells. Our results suggest that MPI cells passaged for <1 mo are highly representative of ex vivo AMs, but lose their AM-like-likeness upon further sub-culturing. Their high isolation yield and self-replicating capabilities make them an attractive and accessible AM model for large scale mechanistic studies.

2409 Deoxynivalenol-Activated Caveolae Impair EGFR-Mediated Barrier Protection

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In response to foodborne trichothecene ribotoxins, the intestinal lining undergoes various types of epithelial adaptation or pathologic distress via stress-responsive eIF2α kinase signaling and subsequent cellular reprogramming. As a vital platform for growth factor-linked adaptive signaling, caveolae were evaluated in deoxynivalenol (DON)-exposed gut. Ulcerative insult enhanced expression of caveolin-1, the main structural component of caveolae, which was positively associated with expression of protein kinase R (PKR), a sentinel kinase in response to trichothecenes. PKR-linked biological responses were simulated in DON-exposed gut models using mice and nematode. Caveolar activation counteracted the expression of wound-protective epidermal growth factor receptor (EGFR) and its target genes, such as chemokines that were pivotal for epithelial integrity in DON-exposed gut. Mechanistic findings regarding trichothecene-associated gut disorder provide crucial molecular evidence for detrimental caveolar actions against EGFR-mediated epithelial protection in patients with ulcerative injuries. This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2018R1A1B3F03014188) and Ministry of Science and ICT (NRF-2019R1A2C1084827).
2410  Biodistribution of Plasmacytoid Dendritic Cells in Nonhuman Primate Lymphoid Tissue


Plasmacytoid dendritic cells (pDCs) are one of the functional subsets of antigen-presenting bone marrow-derived leukocytes, mainly involved in the regulation of the antiviral immune response, mucosal immunity and the development of central and peripheral tolerance. The severity of pDCs infiltration correlates with disease progression as pDCs have been associated with the immunopathology of cancers and autoimmune diseases. Since the immune system of the nonhuman primate (NHP) closely resembles the human, the macaque model provides a unique opportunity for studying pDCs. NHP pDCs exhibit similar morphology and phenotype HLA-DR+CD11c+CD123+ to their human counterparts. In the previous study, it has been demonstrated that CD303 can be used as a specific marker for pDCs detection in NHP tissues. By using immunohistochemical (IHC) and immunofluorescence (IF) methods. Data showed a direct correlation of CD303+ pDCs tissue distribution when measured via flow cytometry (FC) and IHC. While CD123 is still one of the critical antigens for identifying pDCs via FC, its expression on endothelial cells makes it a less favorable marker for histopathological evaluation using IHC or IF. Therefore, our study aims to understand the biodistribution of pDCs in NHP lymphoid tissues in the context of pre-clinical toxicology studies. Bone marrow and peripheral lymphoid tissue, including spleen, regional (mandibular, mesenteric, bronchopulmonary) lymph nodes (LN) and mucosal associated lymphoid tissue (MALT) containing oropharyngeal (tonsil), small intestine (ileum) and respiratory tract (bronchial) inflammation were collected from naive cynomolgus monkeys (Macaca fascicularis). IHC and IF were performed on formalin-fixed paraffin-embedded tissue sections from the same animal. Immunophenotyping of unfixed tissue (bone marrow, spleen and tonsils) leukocytes was performed by flow cytometry. pDCs constitute ≤ 1% of CD45+ lymphocytes in tissues with highest proportions in bone marrow compared to regional LN and tonsils. Both FC and IHC results indicate lower pDCs proportions in peripheral lymphoid tissue compared with bone marrow. In IHC higher relative proportions of pDCs were noted in spleen and tonsils, compared to regional LN and other MALT (ileum and lung) in naive monkeys. Therefore, characterization of pDCs biodistribution in naive NHP lymphoid tissue can help understand basal levels of healthy tissue.

2411  Investigating the Sex-Specific Effects of Arsenic Exposure on the Risk of Influenza Infections


It is estimated that an average, eight percent of people living in the United States will get infected with influenza virus each season. Epidemiological studies consistently report that environmental immunotoxins, such as arsenic, increase susceptibility to respiratory infections, including the H1N1 influenza A virus (IAV). We previously observed that IAV-infected female mice exposed to arsenic experienced greater morbidity and mortality compared to males. Specific mechanisms that contribute to these observed sex differences in immune responses remain unknown. We hypothesize that sex steroid hormones contribute to the arsenic-related sex-specific immune response against IAV, leading to an exacerbated immune response in females. To investigate this hypothesis, we exposed ovariectomized female mice, with or without intranasal IAV infection, to 0, 10, or 100 ppb of sodium (meta) arsenite in drinking water. Viral titers using median tissue culture infectious dose, and cytokine levels as assessed by ELISA, and cytokine measurements using a multiplex Lumienx assay were performed. While there is a reduction in body temperature in ovariectomized mice post-infection compared to the control group, the absence of sex hormones did not significantly alter the arsenic-related disease severity. Furthermore, our preliminary findings suggest that the exposedarsetic response in females may not be driven solely by sex hormones, as other mechanism(s) may contribute to the observed sex differences. Therefore, we are conducting extensive flow cytometry analysis to determine how arsenic alters different immune cell types in the lung, spleen, and lymph nodes across exposure groups. We will also determine whether arsenic alters the expression of immune-related X-linked genes. This research will further elucidate the mechanism by which arsenic impairs immune function, altering cytokine levels and cell function differently in females, and help explain the observed sex differences during IAV infection.

2412  A9-Tetrahydrocannabinol (THC) and Cannabidiol (CBD) Reduce MCP-1 Production in Interferon Gamma (IFN-γ)-Stimulated u251 Human Astrocytes


The implementation of combined antiretroviral therapy (cART) has altered the prognosis of HIV infection to a manageable chronic disease; however, the aging HIV positive population has an increased incidence of HIV associated neurocognitive disorders (HAND). It’s hypothesized that leaky gut syndrome associated with HIV infection and HAND is due to alterations in the intestinal microbiome and abnormally high levels of cytokines. Evidence suggests that cannabinoid receptors (CB1 and CB2) and CD8+ T cells, that migrate through circulation and cross the blood brain barrier (BBB), Once in the brain activated immune cells release inflammatory factors (e.g., IFN-γ) that interact with central nervous system cells, such as astrocytes, contributing to HAND pathogenesis. Cannabinoids such as THC and CBD from Cannabis sativa have been proposed as potential therapeutics for HAND because of their ability to cross the BBB and assert anti-inflammatory effects. To mimic an inflammatory state induced by CD8+ T cells and assess the anti-inflammatory effects of cannabinoid treatment, a human glioblastoma line, U251 cells, were treated with 10 ng/mL of IFN-γ in combination with either THC or CBD (0.1, 1, or 10 μM). Culture supernatants were collected for cytokine analysis using a multiplex Luminex assay were performed. While there is a reduction in drinking water. Viral titers using median tissue culture infectious dose, and cytokine analysis revealed statistically significant differences in MCP-1 production by U251 cells at 24 hours. IP-10 and IL-6 responses were not modulated by cannabinoid treatment, but the 10 μM treatment of CBD significantly reduced MCP-1 production. 48 hours post-IFN-γ treatment IL-6 production returned to background, MCP-1 production was reduced, but the number of IP-10 producing cells was comparable to 24 hours. However, treatment with THC and CBD were not able to modulate the MCP-1 or IP-10 response at this time. At 72 hours post-IFN-γ treatment, MCP-1 production returned to background while the IP-10 response was unaffected by THC or CBD treatment. These results suggest that CBD and THC can reduce MCP-1 production by U251 cells in response to IFN-γ stimulation, which may be indicative of the potential anti-inflammatory role that THC and CBD may play in reducing IFN-γ induced neuroinflammation. Supported by NIH R01 DA047180.

2413  T Cells from Smokers Show Signs of Aging and Immune Dysfunction

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As a risk factor for human diseases, the global disease burden attributed to tobacco smoke exposure remains substantial. Using mass cytometry and single-cell RNA sequencing (scRNAseq), we compared immune cells from the blood of healthy smokers and nonsmokers to find that smokers’ cells exhibited characteristics of aging and immune dysfunction. A rare subpopulation of CD16+ T cells, which exhibited transcriptional signatures of senescence combined with high cytolytic potential, was expanded in smokers. Consistent with accelerated aging, smokers’ CD8 T cells were biased toward differentiated cell types and smokers had reduced naïve cell proportions. DNA methylation-based models (N = 131) showed significant associations between smoking dose (reduced 4-2HRR methylation) and shortened telomeres (p = 0.0002, r2 = 0.11) and age acceleration (p < 0.00009, r2 = 0.11). Accumulation of terminally differentiated T cell subsets and reduction in naïve T cells can lead to attenuated immune responses when clonal expansion results in loss of diversity in antigen recognition within T cell populations. The TCR repertoire reflects the immunological memory and exposure history of an individual. Overrepresentation of shared TCR clonotypes among smokers’ CD16+ CD8 T cells could indicate clonal expansion. Clonal diversity of naïve and central memory T cells is necessary to develop and maintain immunological memory. Therefore, a loss of diversity in these subsets would suggest impaired functional capacity in smokers’ immune systems. To understand the possible role of clonal expansion and TCR diversity within T cells of smokers, we profiled T cell receptor (TCR) clonotypes in T cell subsets from smokers and nonsmokers using single-cell TCR sequencing. The premature aging of CD8 T cells, which have an effector role in adaptive immunity, provides a potential explanation to the paradox that exposure to tobacco smoke is associated with both impaired immunity and increased risk of inflammatory diseases. As such, these findings may provide a link between immune dysfunction and smoking-mediated diseases.
Experimental autoimmune encephalomyelitis (EAE) is an autoimmune disease model of multiple sclerosis that can be induced in mice using the myelin oligodendrocyte glycoprotein (MOG) peptide. Perturbation of the pathological process of EAE involves production of antibodies that can recruit cytotoxic cells to destroy MOG-expressing cells, contributing to myelin destruction. Previously we showed that TCDD inhibited MOG-specific IgG and decreased disease at 18 days (end-stage disease). In an in vitro model, we also showed that TCDD preferentially inhibited IgG1 cell surface expression. Thus, the purpose of this study was to characterize the effects of TCDD on the IgG1 subclass of IgG to further investigate the mechanism behind TCDD’s known immune suppression. We hypothesized that TCDD would suppress IgG1 production in vivo and in vitro. In a recent EAE study, TCDD significantly suppressed MOG-specific IgG1 in the serum at end-stage disease. ELISA analyses showed that TCDD modestly inhibited total IgG in mouse spleenocytes or purified B cells stimulated with LPS or LPS plus IL-4 for 4 days. Additional ELISA analyses showed that TCDD inhibited IgG1 production in spleenocytes or purified B cells stimulated with LPS plus IL-4 at various times over the 4-day culture, but there was no significant effect on IgG1 production in bone marrow at 2 days post stimulation. Together these data show that a sensitive target of suppression by TCDD is the IgG1 subclass of IgG, which was inhibited in vivo in the EAE model and in vitro. While TCDD could not be developed as a therapy for autoimmune disease, these studies provide insights for the mechanisms by which AhR ligands are immune suppressive. Supported by NIH R15 ES027650.

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The mechanism of action of many toxicants involves inflammatory responses which can exacerbate tissue damage. It is clear that deaths from some infectious diseases, including COVID-19, result from excessively aggressive inflammatory responses. Thus, toxicants that also exacerbate or which inhibit inflammation would be expected to alter the course of such infections. With this in mind we have developed a panel of 12 labels that can be used to characterize M1 (inflammatory) and M2 (wound healing) macrophages and to thereby assess the effects of toxicants on these critical responses. We report here the labeling of the mouse macrophage cell line RAW 264.7 with the following labels/antibodies followed by assessment by flow cytometry to identify M1 and M2 macrophage types and to assess the effects of toxicants: live/dead viability dye, F4/80, Arginase 1, iNOS, TLR4, CD86, VEGF, CD14, TNF-alpha (surface and internal), CD206, and MHC Class II. Titration of antibodies and assessment of reproducibility of this number of labels is difficult and has not been previously used to assess effects of toxicants. We have identified clear distinctions between M1 macrophages (polarized by exposure to IFN-gamma and LPS) and M2 macrophages (polarized by exposure to IL-4) using these markers, and we suggest this is a useful approach to identify the effects of infectious agents and toxicants on inflammatory responses.

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Nevirapine is a non-nucleoside reverse transcriptase inhibitor used in the treatment of HIV infection. Its use is limited due to its risk of causing idiosyncratic drug reactions (IDRs) such as idiosyncratic drug-induced liver injury and skin reactions. Multiple lines of evidence including HLA associations suggest that these reactions involve an adaptive immune response, and it is likely that the requirement for a specific HLA/T cell receptor combination is what makes IDRs idiosyncratic. However, an adaptive immune response requires a prior innate immune response, which is unlikely to be idiosyncratic or symptomatic. We performed the present study to characterize early changes in immune cells in mice caused by nevirapine that precede an IDR. Male C57BL/6 mice were administered nevirapine by oral gavage for three days and sacrificed three hours following the final dose. Flow cytometry was used to enumerate leukocyte subsets in blood, spleen, and inguinal lymph nodes (ILN). Treatment with nevirapine resulted in decreased spleen and ILN weights, and also decreased cell counts in blood, spleen, and ILN. Monocytes, neutrophils, eosinophils, and natural killer cells were decreased in all three organs as a proportion of leukocytes. B cells were also decreased in ILN and blood but not spleen. The proportion of T cells appear to be increased in all three organs and most clearly in blood; however, absolute counts show that T cells were decreased less than other cell types in the spleen and ILN of treated mice, while T cell numbers did not change in blood. Annexin V analysis showed increased cells (across all cell types) undergoing late apoptosis and necrosis in blood and spleen, and the recovery of the mice did not show resolution of these cell populations. These data show that nevirapine causes clear decreases in multiple leukocyte subsets in multiple organs after three days of treatment, which may suggest toxicity to immune cells or perhaps a means of immunosuppression to prevent the immune system from unnecessarily attacking host cells. Indeed, leukopenia has been observed clinically thereby contributing to the causation of IDRs, so this may be an important feature of drugs that cause IDRs. Further studies are required to understand the mechanism causing the decrease in leukocytes. Understanding the effects of drugs that cause IDRs on the immune system is important for the treatment and prevention of IDRs in order to use these drugs more safely in patients. Funded by CIHR and scholarships from NSERC and AFPC/Merck Canada Inc.

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Inhalation of crystalline silica (cSiO2), a known trigger of autoimmune disease, elicits alveolar macrophage (AM) death and pulmonary inflammation. Inefficient removal of resultant cell corpses via phosphatidylserine (PS)-mediated phagocytic process known as effecrocytosis (EF), can increase accumulation of observed antigens thereby contributing to the causation of autoimmunity. Consumption of omega-3 docosahexaenoic acid (DHA), found in fish oil, markedly ameliorates cSiO2-triggered autoimmunity in lupus-prone NZBWF1 mice. Here we tested the hypothesis that DHA influences EF of toxicant-induced cell corpses via esterification of cell membrane PS with oxidizable DHA. ASC-transfected RAW 264.7 murine macrophages (RAW-ASC, target) were pre-incubated with Veh or 50 μM DHA, i) labeled with pHrodoRed dye, and iii) killed by incubation with cSiO2 or exposure to UV light. Resultant target corpses were then incubated with Vehicle(Veh) or lipoxygenase-associated phospholipase A2 (Lp-PLA2) for 1 h to selectively hydroxylize oxidized PS (PSox) species on RAW-ASC corpses. Oxidation and removal of PS esterified DHA on target cells were confirmed by LC/MS. In vitro studies, treated RAW-ASC corpses were then co-cultured for up to 1 h with CSFC green-labeled Max Planck Institute (MPI) cells (effector), an AM surrogate, that were pre-incubated with Veh or DHA. Treated target cells wereophytyngually instilled into C57BL/6J mice. Co-cultured MPI effector cells and cytospin slides of AM recovered in BALF were analyzed for EF by microscopy. Pre-incubation of target cells with 50 μM DHA prior to death induced by cSiO2, or UV significantly enhanced EF by MPI cells. In contrast, pre-incubating effector cells with DHA did not affect the engulfment of target cell corpses. Selective removal of the outer surface of UV induced target corpses by Lp-PLA2 resulted in suppression of EF only in DHA preincubated cells and this suppression was blocked by inhibitor of Lp-PLA2, Darapladib. Similarly, pre-incubation target cells with DHA pre-incubation significantly enhanced EF by AM and removal of PSox from target cells suppressed EF in vivo. Taken together, these novel findings indicate that DHA esterification of PS increases the rate of oxidized PS-mediated EF by AM, with the net effect of reducing the accumulation of corpses capable of eliciting autoimmunity.

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Persistent AhR signaling modulates multiple branches of human hematopoiesis by mechanisms that are not well understood. Single cell transcriptomics was employed to study the effect of AhR activation by 1 μM 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on early stages (14 days of culture) of hematopoietic development. An in vitro culture system was used that favors B lymphocyte development from human cord blood derived CD34+ hematopoietic stem and progenitor cells (HSPCs) but also allows development of precursors belonging to other hematopoietic lineages. The aim was to determine how...
persistent AhR signaling modulates gene expression in differentiating HSPCs and regulates development of different hematopoietic cells. Cell clusters were generated based on gene expression using machine learning algorithms and annotated based on characteristic hematopoietic markers. Cells belonging to plasmacytoid dendritic cell (pDC) and megakaryocyte-erythroid (MEK) lineages were drastically reduced with reductions in pDC and MEK progenitors in TCDD-treated group being 11% and 32% of that of vehicle (0.02% DMSO) on Day 14. However, monocyte and neutrophil progenitor populations showed an increase of 18% and 15% respectively, suggesting that persistent AhR activation skew hematopoiesis towards particular myeloid lineages. Flow cytometric analysis confirmed reduction in common lymphoid progenitors, early, and Pre-B cells as well as a decrease in pDCs (15% of vehicle) with TCDD treatment at Day 14. Differential gene expression analysis revealed genes critical for pDC development (SPIB, TCF4, BCL11A) and hematoglobin-associated genes were downregulated (log fold change difference >0.5; p value <0.01). Genes associated with ribosomal protein synthesis were reduced in lymphoid clusters indicating inhibition of ribosomal protein production machinery and perhaps cellular proliferation rates. Functional enrichment analysis of differentially expressed genes showed an increase in inflammatory pathway response in most cell types. Overall, this study reveals how persistent AhR signaling modulates distinct gene expression programs in differentiating HSPCs and regulates development of different hematopoietic lineages. Supported by NIH P42 ES049911.

**2419 Comparison of Sensitization Potentials between Silver Nanoparticles and Silver Ions Using Monocytic Cell Line THP-1**


Although silver nanoparticles (AgNPs) were widely used in cosmetics and daily necessities based on their excellent antimicrobial activity, the effects of AgNPs on human health, especially immune system, have not been fully elucidated. In particular, it was not clear whether the AgNPs or the silver ions (Ag⁺) released from the AgNPs have sensitization potentials. In this study, in order to investigate the sensitization potentials of AgNPs and Ag⁺ ions, activation of the antigen-presenting cells after treatment with AgNPs and silver ions were evaluated. Dispersion of AgNPs with 10 nm diameter were exposed to human monocytic cell line THP-1 cells for 24 h, and cell viability and the expression level of CD54 and CD86 were measured using flow cytometer according to the human Cell Line Activation Test (h-CLAT) protocol (OECD TG442E Annex I (2018)). The similar measurements on silver nitrate (AgNO₃) were also performed to compare the ability to activate the antigen-presenting cells of AgNPs and Ag⁺ ions. As a result, the expression level of CD54 and CD86, markers of activation of antigen-presenting cells increased and exceeded the positive criteria for h-CLAT (CD54: >200%; CD86: >150%) after treatment with both AgNPs and AgNO₃. Interestingly, the EC150 (a concentration at which CD86 exceeded the criteria) and EC200 (a concentration at which CD54 exceeded the criteria) of AgNO₃ were much lower than those of AgNPs. Furthermore, the results indicated that both AgNPs and Ag⁺ ions have the ability to activate antigen-presenting cells, however, Ag⁺ ions showed higher activation potentials than silver nanoparticles. Then, cellular uptake of AgNPs and Ag⁺ were quantified by using inductively coupled plasma atomic emission spectroscopy (ICP-AES). When AgNPs and Ag⁺ were exposed to THP-1 cells at a concentration showing a similar viability (CV75), a larger amount of AgNP and Ag⁺ were uptaken in the cells than Ag⁺ ions. It is possible that cells uptake AgNP, migrates to lymph, and released Ag⁺ ions activates antigen-presenting cells. In conclusion, evaluation of antigen-presenting cell activation ability using THP-1 revealed that Ag⁺ ions showed higher activation potentials than silver nanoparticles. It was also suggested that evaluation of antigen-presenting cell activation ability using THP-1 cells is also expected as a method for evaluating the sensitization potentials of other nanomaterials.

**2420 Immunotoxicological Investigations of PFAS Found in North Carolina Drinking Water Sources**

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Novel per- and polyfluoroalkyl substances (PFAS) were recently identified in surface waters used as drinking water sources within North Carolina. Some of these novel PFAS have replaced longer carbon chain compounds such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate, but some appear to be degrading to transformation or by-products of fluoropolymer production. Those discovered included the perfluorooether acids (PFEAs) perfluoro-2-methoxyacetic acid (PFMOAA), perfluoro-2-methoxypropanoic acid (PFMOAPA), and perfluoro-4-methoxybutanonic acid (PFMOBA). The toxicity of PFOA has been well-characterized in animal models and observed in epidemiological studies of humans; however, little toxicological data appear to exist in publicly available literature for PFMOAA, PFMOAPA, and PFMOBA. Therefore, the present studies sought to describe signs of toxicity and immunotoxicity following 30-days of exposure. Young adult male and female C57BL/6 mice were orally exposed daily for 30 days to PFMOOA (0, 0.00025, 0.025, or 2.5 mg/kg); PFMOAPA or PFMOBA (0, 0.5 mg/kg, 5, or 50 mg/kg), or PFOA (7.5 mg/kg) as a positive control. Endpoint included in-life observations, organ weights, immunophenotype of lymphoid organs, liver peroxisome proliferation, natural killer (NK) cell cytotoxicity, and evaluation of the T cell-dependent antibody response (TDAR). At doses administered, terminal body weights, liver, spleen, or thymus weights did not differ among doses. Shifts in immune cell populations were observed in spleen and thymus of both sexes in response to PFMOOA and in the thymus of females in response to PFMOBA. Numbers of splenic B and NK cells were increased in males by ~83% and ~97%, respectively in response to PFMOOA at 2.5 mg/kg. PFMOBA induced a ~50% increase in peroxisome proliferation in females from 50 mg/kg, whereas PFMOAA induced a ~25% increase in peroxisome proliferation from females at 2.5 mg/kg. Exposure to PFMOAPA did not affect the measured parameters at administered doses. Exposure to PFMOAPA or PFMOBA did not alter NK cell cytotoxicity (PFMOAA was not evaluated for NK cell cytotoxicity) or the TDAR at doses administered. Our results indicate that these “understudied” PFAS discovered in North Carolina have toxicological potential but require additional investigation across endpoints and species, including humans, to understand their health effects via drinking water exposure.

**2421 The Nr2F2 Activator, tBHQ, Inhibits Early NK Cell Responses to Influenza**


Influenza virus is estimated to cause millions of illnesses and thousands of hospitalizations and deaths yearly in the United States, making influenza a major public health concern. It is well known that an early natural killer (NK) cell response against influenza is vital for effective viral clearance. tert-Butylhydroquinone (tBHQ) is a widely used food preservative with known immunomodulatory activity. Our lab has shown tBHQ to negatively impact NK cell activation, effector function, and maturation ex vivo. However, little is known regarding the effects of tBHQ consumption on NK cells in vivo, specifically in response to influenza infection. In the current study, we examined whether the consumption of tBHQ would impair NK cell response two and three days after primary influenza virus infection. Female C57Bl/6j mice were fed a diet containing either 0.0014% tBHQ or control diet 10 days prior to infection. Mice were intranasally infected with influenza A/PuR/8/34 (H1N1), and at day two and three post-infection, lungs were collected and processed for analysis by flow cytometry, qPCR, and ELISA. Mice exhibited similar weight loss throughout infection regardless of their respective diet. There were no statistical differences in the infiltration or maturation of NK cells in the lungs between the groups at day two of infection. At day three, tBHQ modestly decreased the percentage of NK cells in the lung, and NK cells adopted a more immature phenotype. Notably, tBHQ significantly reduced expression of FaS and production of IFNγ in NK cells compared to those on a control diet at day two. NK cell cytotoxicity decreased with tBHQ at day three, as demonstrated by reduced CD107a expression. Likewise, induction of the effector genes was decreased in the lungs of mice on a tBHQ diet at days two and three of infection. Taken together, the data suggests the food additive, tBHQ, to negatively impact early NK cell responses to influenza infection by inhibiting FaS and CaD107a expression and IFNγ production. Furthermore, tBHQ impaired the induction of genes associated with NK cell effector function in influenza-infected mice. This study was funded by NIH grant R01 E024966.

**2422 Aryl Hydrocarbon Receptor Regulation of T Follicular Helper Cells during Respiratory Viral Infection**

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Environmental factors are key modulators of immune function. However, the mechanisms by which immune cells sense environmental cues, and integrate them into specific immune responses are not well understood. The aryl hydrocarbon receptor (AhR) is one means by which environmental signals regulate immune responses. The AhR is a ligand-regulated transcription factor that binds structurally diverse molecules, including certain pollutants, dietary factors, and chemicals from microorganisms. Epidemiological and animal studies demonstrate that AhR-binding compounds alter adaptive immune responses, but the cellular components and governing mechanisms are not fully defined. For instance, exposure to AhR ligands affects antibody responses to...
C. E. Rockwell1.

additive

posed mice. To investigate the role of Nrf2 in these effects, Nrf2-floxed mice

monia. Following heterosubtypic infection, mice on tBHQ-containing diet had
tor function as seen by reduced expression of cytotoxicity markers, FasL

number of influenza-specific CD8+ T cells in draining lymph nodes compared

ies demonstrate that skin-sensitization with OVA induced a markedly higher

was to determine the effect of the food preservative
ter BHQ) on anaphylaxis in a mouse model of food allergy. Our preliminary stud-

tor function of CD8+ T cells during primary infection was diminished by tBHQ

fed mice had a reduced number of CD8+ T cells in the lungs and a reduced

expression of BCL6, an essential transcription factor for Tfh cell differentiation.

immune challenges; a process for which T follicular helper cells (Tfh cells)
critical. We recently discovered that AhR activation with several different

effect of CD8+ T cells during primary infection was diminished by tBHQ

Influenza infections cause hundreds of thousands of hospitalizations in the

The present results demonstrate that Skimune is a highly sensitive assay for

P2423 The Synthetic Food Additive, tert-

Butyrolactone, Impairs the Effector T Cell Response to Influenza Virus through a

Nrf2-Dependent, T Cell-Intrinsic Mechanism


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Influenza infections are a major public health concern, globally impacting over 3 billion people each year. Despite the availability of vaccines, influenza infections cause hundreds of thousands of hospitalizations in the United States each year. Recent studies have shown that the synthetic food additive tert-butylhydroquinone (tBHQ), commonly used as a food preservative, can exacerbate allergic responses. In this study, we investigated the effect of tBHQ on the immune response to influenza infection.

Our results demonstrated that tBHQ treatment during influenza infection diminished the activation of CD8+ T cells, as evidenced by reduced expression of cytotoxicity markers such as FasL. This reduction in CD8+ T cell function was associated with a decrease in the number of influenza-specific CD8+ T cells in the lungs and a reduction in the percentage of Th2 cells. Furthermore, recipients of Nrf2-deficient CD4 T cells showed a greater drop in body temperature in mice receiving wild-type, but not Nrf2-deficient, CD4 T cells, suggesting these effects are dependent on Nrf2.

We conclude that tBHQ exacerbates allergic response in ovalbumin (OVA) sensitized mice through activation of Nrf2. These findings provide new information about how the AhR transduces environmental signals to influence the humoral immunity to a common respiratory pathogen. Given that exposure to AhR-binding pollutants correlates with greater severity of infections and reduced antibody responses to common vaccines, better understanding of the mechanisms that control Tfh cell differentiation and function has broad reaching impact on immune defenses and immune-mediated diseases.

P2425 Skimune, an In Vitro Human Skin Explant Assay to Predict Adverse Immune Reactions Associated with Cytokine Release


To aid preclinical prediction of immune reactions associated with cytokine release caused by LMW biopharmaceuticals and biologics, Alcyomics developed an in vitro human skin explant assay (Skimune). Peripheral blood mononuclear cells (PBMC) from CD14 positive healthy volunteers were differentiated into mature dendritic cells, which were then treated for 24 hours with the LMW compound. CD14 negative cells containing T cells were then added and co-cultured for 6 days. The cells were then assessed for T cell proliferation using tritiated thymidine uptake assays and supernatants collected for IFNγ analysis using the MSD platform. In addition, a 4-mm skin biopsy from the same healthy volunteer was dissected and the activated cells from the collected culture added and further cocultured for 3 days. Then, the skin sections were formalin fixed and stained using haematoxylin and eosin and graded from Grade I-IV in order of severity. Twelve LMW drugs associated with T cell-mediated hypersensitivity reactions in the clinic (abacavir, amoxicillin, carbamazepine, diclofenac, lomotrigine, latapinth, lumiracoxib, nevirapine, oxifacon, phenytoin, propranolol, sulfamethoxazole) were compared with 5 drugs with rare or no clinical reports (acetaminophen, cimetidine, flecainide, metformin, verapamil). Results for T cell proliferation (r=0.6 p<0.01), IFNγ release (r=0.51 p<0.04) and Skimune* (r=0.77, p<0.001) between both groups were statistically significant. The Skimune method was modified for the testing of biologics, where unseparated healthy volunteer PBMCs were added to the biologic (1μg/ml) and autologous skin section, and co-cultured for 3 days prior to the skin being processed as described above. 17 biologics were tested. Biologics known to cause adverse reactions such as Campath and OKT3 as well as the TG1412 analogue gave a Grade II or III histopathological skin response in 70% to 90% of individual tests. There was a positive correlation between adverse clinical outcome and the response in Skimune* (r=0.815, p<0.0001). The present results demonstrate that Skimune is a highly sensitive assay for predicting drug-induced allergic immune reactions involving acute cytokine release.

P2426 Prenatal Cadmium Exposure Alters Proliferation in Mouse CD4+ T Cells via LncRNA Snhg7

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Cadmium (Cd) heavy metal exposure is of concern due to its long half-life and its association with numerous health issues including pregnancy and reproductive disorders, developmental toxicity, and cancer. Previous studies have shown that Cd affects the immunological responses in Cd-exposed parents. This study identified genes and pathways altered by prenatal Cd exposure in mouse primary CD4+ T cells. Using RNA-Seq, the long noncoding RNA, IncSnhg7, was shown to increase expression level in primary Cd4+ T cells of both male and female offspring after ex vivo stimulation with anti-CD3/CD28. LncRNAs are PoF. Cd T cells infected with 200 nM nucleotides and lack protein encoding potential. They alter gene expression through various mechanisms including micro-RNA sponging, histone and
chromatin modifications, and regulating protein translation. LncSnhg7 sequesters the microRNA miR-34a resulting in increased expression of polypeptide N-acetylgalactosaminyltransferase 7 (GALNT7) and increased proliferation in cancer cell lines. The hypothesis of this study is that lncSnhg7 functions to sequester miR-34a in CD4+ T cells, thereby controlling the production of GALNT7 and proliferation in these primary cells. LncSnhg7 expression increased in CD4+ T cells in offspring exposed to prenatal Cd and correlated with increased expression of GALNT7 protein, but not mRNA transcripts. To assess whether increased GALNT7 correlates with increased proliferation of CD4+ T cells, cells were stained with Cell Trace Violet and stimulated in vitro for 5 days. FCS express software was used to calculate the division index (the average number of cells that a dividing cell eventually became). T cells isolated from Cd-exposed offspring divided significantly more than those from control offspring (Division index= 16.2 versus 9.9, respectively) (p<0.05). No differences were observed between control and Cd exposed offspring in the ability of T regulatory cells to suppress the proliferation of CD4+ T cells. Apoptosis was not altered by Cd exposure as measured by Caspase 3/7 and Annexin V/7AAD assay. These results provide further insights into the molecular mechanisms of immune dysregulation that are a consequence prenatal cadmium exposure.

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**The Response of Natural Killer Cells, B Cells, and T Cells to Influenza Is Diminished In Vitro in the Presence of Arsenic Trioxide**

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Arsenic is a common environmental toxicant in multiple areas within the United States, particularly in the Great Lakes region, the north east and the south west. People relying on individual water supply sources, such as well water, are often exposed to higher concentrations of arsenic than recommended by the EPA. Arsenic is a known activator of Nuclear factor erythroid 2-related factor 2 (Nrf2). Nrf2 is a stress-inducible transcription factor present in mammalian cells. It is a key regulator for both cytotoxic protective genes and immunosuppressive effects in the context of inflammation. Nrf2 is constitutively bound to its cytosolic repressor protein Kelch-Like ECH-Associated Protein 1 (KEAP1). Upon activation, unbound Nrf-2 translocates to the nucleus to induce Nrf2 target genes. In this study, we evaluated the role of As2O3, in modulating ex vivo immune responses upon exposure to H1N1 influenza using peripheral blood mononuclear cells (PBMCs) isolated from whole blood. PBMCs were purified and treated with either 1 μM As2O3 or PBS and challenged with H1N1 influenza virus. 96 hours later, cells were fluorescently labeled and assessed for T-cell, NK-cell and B-cell specific activation. The data indicate that As2O3 causes a decrease in CD4 T cell activation. Primary human T cells treated with arsenic trioxide and challenged with influenza A virus showed reduced viability, a reduction in the population of memory cells, and reduced IFNγ and granzyme B production. Other effector molecules expressed by CD8 T cells, such as CD107a, were not diminished by As2O3. Taken together, the data suggest that arsenic trioxide impairs the activation and production of effector cytokines by human immune cells to H1N1 influenza. This work was supported by NIH grants ES024966 and GM092715.

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**2429 Alveolar and Interstitial Macrophages Are Activated in a Model of Pulmonary Fibrosis**


Interstitial lung disease (ILD) is an increasingly prevalent disease in the United States, with 34,000 new cases diagnosed per year and the most common diagnostic group to receive lung transplantation. There are multiple etiologies including systemic disease, environmental exposure, consequences of chemotherapy, or idiopathically. ILD is characterized by chronic inflammation in the lung interstitium leading to fibrosis and restrictive lung disease. Currently, there are no effective treatments that prolong survival other than transplantation. Pulmonary macrophages play a significant role in lung injury and repair, however, their involvement in ILD is less clear. In this study we used a transgenic adenine (IP) knock-in of intrapulmonary bleomycin to generate a model of sustained pulmonary fibrosis. C57BL6/J mice were injected IP with 0.8μl bleomycin or PBS control every 3 days up to 15d and then were sacrificed at 21 or 40d. Mice administered IPB lost significantly more weight than control and had increased lung leak at 21 and 40d (BAL protein 80.6(control) vs 79.4(1*21d) vs 65.5(μg/[40d])). IPB resulted in a significant increase in cell invasion (167±5.6 vs 236±15.6* vs 223±16.0* cells/40x window), alveolar wall thickness (2.0±1 vs 3±0.1* vs 3±0.1*μm) and a loss of alveolar space (66±1.4 vs 49±1.2* vs 53±3.3%). This consolidation was confirmed as fibrosis by trichrome staining at 21 and 40d. Flow cytometric analysis of BAL indicates a loss of alveolar macrophages (CD45+/Siglec F+4/40d+) with IPB (95±0.2 vs 10.4*1 vs 25±6.5%*) and increased migratory alveolar macrophages (CD11c+/CD11b+) (0±0.1 vs 44±8.8* vs 43±3.9%). Interstitial cells were prepared from a lung digest. CD206 expression was increased in interstitial macrophages (CD45+/Siglec F+/4/40d+) with IPB (3±0.3 vs 18±1.9* vs 18±3.6%*) in IPB mice. Flow analysis of the mesenchymal cell population (CD45-/CD31-/SCA-1+) showed increased expression of CD44 and CD90 with IPB at 40d (4±1.8 vs 3±0.1 vs 64±3.7%*), consistent with fibrosis. These findings suggest IPB results in sustained pulmonary fibrosis and is associated with significant shifts in both the alveolar and interstitial macrophage phenotypes. These data indicate that inhibition of macrophage maturation may represent a mechanism to reduce fibrosis. (p<0.05 when compared with control (*) or 21d (##)).

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**2430 Evaluation of Physiologically Relevant Aryl Hydrocarbon Receptor Ligands on Antibody Expression in a Human B Cell Line Model**

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The aryl hydrocarbon receptor (AhR) is a transcription factor that affects immune cell differentiation and function in animal models. AhR may serve as an environmental sensor since it not only binds to toxicants such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) but also to endogenous, dietary and bacterial ligands such as 6-formylindolo[3,2-b]carbazole (FICZ) and indole. Indole is a human specific AhR ligand that is relatively abundant in the gut. In animal studies, different AhR ligands have produced different and even opposite functional effects on T cell subtypes, which may be due to transient vs. persistent activation of the AhR. The objective of this study was to determine if AhR ligands differentially modulate antibody expression and secretion in a human B cell line (CL-01). Previous results have demonstrated inhibition of IgG but not IgM secretion by TCDD (30 nM). Current preliminary results suggest that higher concentrations of FICZ (100 nM) and indole (100 μM) inhibit both IgM and IgG secretion. These results suggest that antibody expression may be less sensitive to endogenous ligands than TCDD and different antibody isotypes may be differentially affected by ligands. Ongoing studies will determine the sensitivity of transcriptional expression of individual antibody isotypes (i.e. IgM, IgG1, IgA1-2, IgE) to AhR ligands and whether physiologically relevant AhR ligands induce different antibody isotype profiles.
2431 How Sex-Specific Arsenic Immunomodulation Increases Disease Risk

More than 2 million people worldwide are at risk of exposure to inorganic arsenic (iAs). Ingestion of contaminated drinking water and food, iAs is a potent carcinogen and immunotoxicant which causes increased risks of cancer other immune-related diseases. However, it is unclear what the causational mechanisms are. We hypothesize that iAs causes a sex-specific immune imbalance leading to the on the one hand increased infectious disease risk, while on the other hand contributing to a tumor-promoting microenvironment. Here we reviewed the effects of iAs (sodium meta-arsenite) on macrophages, innate immune cells known for their antimicrobial functions (M1) as well as wound-healing and tumor-promoting capabilities (M2). We studied macrophages cultured from bone marrow harvested from 8-12 week old adult C57BL/6 mice, that were dosed with different levels of iAs (0.0001 - 1 μM) during differentiation, and stimulated with either M0 (M-CSF alone), M1 (LPS and IFN-γ) or M2 (IL-4 and IL-13) polarizing ligands. We observed sex- and dose-specific suppression of nitric oxide (NO), iNOS and cytokine production, phagocytosis and differences in cell surface expression (incl. MHCII, CD86, Arginase, CD163), as assessed by flow cytometry in activated bone marrow-derived macrophages (BMDMs) in vitro treated with varying iAs (0.0001 - 1 μM) either during or after macrophage differentiation. Moreover, signaling lipid analysis revealed a skewing toward an M2 tumor-promoting phenotype. In line with these findings, a lung cancer cell line which was co-cultured with iAs-exposed macrophages showed increased proliferation compared to when cultured with unexposed macrophages. iAs-exposing macrophages displayed increased migration toward lung cancer cell conditioned media, which may in part be attributed to their increased expression of chemokine receptor CCR2. Importantly, we observed similar suppression of the proinflammatory M1 phenotype and skewing toward an M2 phenotype in activated lung macrophages in vitro treated with either iAs (0.0001 - 1 μM) or LPS. However, this was not observed in vitro treated with 100 ppb iAs. RNAseq data is currently generated for mechanistic pathway discovery. We are also investigating how these macrophage alterations influence infectious disease risk versus cancer risk. Overall, our data elucidate the different ways how iAs can influence disease risk in exposed populations through immunomodulation. NEHS R00ES024808 (FS), T32ES07141 (KR, EI) and ST32HL07534-37 (SS).

2432 AhR-Mediated Transcriptional Regulation of the Human Immunoglobulin hs1.2 Enhancer
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The 3’IGHRR, an ~17kb transcriptional regulatory region within the human immunoglobulin heavy chain gene (IGH), is thought to be responsible for the transcription of the IGH locus, which is essential for antibody production. The 3’IGHRR contains the hs1.2 enhancer which is polymorphic in humans and consists of a 53 bp invariant sequence containing transcription factor binding sites, including a potential dioxin response element (DRE) that can be duplicated one to four times. Previous experiments have shown that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) can induce transcriptional activity of the human hs1.2 enhancer alleles via the aryl hydrocarbon receptor (AhR) signaling pathway. The objective of this study is to assess the role of the AhR transactivation domain (TAD) in TCDD-induced hs1.2 enhancer activity and the transcriptional impact of an increased number of invariant sequences. Luciferase reporter plasmids containing one of the four human polymorphic hs1.2 enhancers (a1A, a1B, a1C, or a1D corresponding to one, two, three, or four invariant sequence repeats, respectively) were transfected via electroporation into a human B-cell line (CL-01) expressing an AhR with either a functional or nonfunctional TAD as determined by the ability to induce a reporter plasmid regulated by 6 DREs. In B cells with a nonfunctional AhR TAD, TCDD activated the hs1.2 enhancer in a concentration-dependent manner but the number of invariant sequences did not impact TCDD-induced activation suggesting a TAD-independent activation of the hs1.2 enhancer by AhR ligands. Ongoing studies are evaluating the impact of TCDD on the hs1.2 enhancer alleles in B cells expressing a functional AhR TAD to determine if a functional TAD will increase the sensitivity of hs1.2 enhancer alleles with a greater number of invariant sequences to TCDD-induced activation. Since the polymorphic hs1.2 enhancer has been associated with altered antibody levels and a number of hypersensitivity and autoimmune diseases, these results will provide greater insight in assessing risk by exposure to environmental, dietary, and endogenous ligands of the AhR.

2433 Development of an In Vitro Model of Drug-Induced Toxic Epidermal Necrosis
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Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are rare severe adverse drug reactions with skin blistering and keratinocyte death. We describe an in vitro skin model of TEN and its modulation by the TNF inhibitor etanercept. Patients on immunotherapy for advanced melanoma were recruited upon initial presentation of SJS/TEN or maculopapular exanthem diagnosis on clinical criteria and confirmed by histopathology. Serum samples (10 ml) were collected at the time of diagnosis. Healthy skin biopsies exposed to TNFa (10 ng/ml), SJS/TEN, rash or healthy control sera, were cultured in the absence/presence of etanercept (1 μg/ml). A positive control (damage induced by Anakinra) and a negative control were used in all assays. The test compound response was compared to the negative control. Skin was incubated for 3 days and graded for histopathological damage (grades I-IV) using criteria similar to those used clinically. Skin sections were also reported for the presence of necrosis or intra-epidermal damage. Skin tests in the presence of TNFa showed grade II responses with vacuolisation of the epidermis. A grade III positive response was observed in response to SJS/TEN sera at 10x dilution in the absence of etanercept with intra-epidermal damage and severe necrosis. At 100x dilution of SJS/TEN sera, the skin damage was reduced to grade II response with necrosis and some intra-epidermal damage. This was further reduced in the presence of etanercept, but no etanercept-induced difference in responses was observed at the 10x dilution. Skin tests showed a 100x dilution of TNFa, SJS/TEN sera and etanercept-induced by etanercept. This represents a putative ex vivo model of TEN with potential for utilisation as a nonclinical safety screen and as a tool for investigating mechanisms of SJS/TEN pathogenesis.

2434 RNA-Seq Reveals Disturbance of Key Neurodevelopmental Processes in the Human Neural Progenitor Test (hNPT) by Diverse Neurodevelopmental Toxicants
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There is a pressing need for human-relevant, animal-free testing strategies to assess compound-induced developmental neurotoxicity (DNT). Human stem cell-based assays are valuable tools in this endeavor due to their ability to mimic important cellular differentiation processes that occur during neurodevelopment. The human neural progenitor test (hNPT) is a 10-day protocol in which neural progenitors differentiate into a neuron-astrocyte co-culture. The aim of the study was to characterise the differentiation over time and to find sensitive neurodevelopmental processes in the assay by exposing hNPT to either one of five known or suspected DNT compounds: acrylamide, chlorpyrifos, fluoxetine, methyl mercury or valproic acid. Transcriptomic analysis showed that 7844 genes were differentially regulated in unexposed control cultures (p ≤0.001, FC ≥0.5). Enriched Gene Ontology (GO) terms among upregulated genes included neuronal differentiation, neurite outgrowth, axon guidance, synaptogenesis, and synaptic transmission of glutamatergic and GABA-ergic neurons. Exposure to either of the five compounds at concentrations resulting in a 5% cell viability reduction resulted in regulation of unique combinations of GO-terms relating to neuron proliferation, neuronal and glial differentiation, axon development, synaptogenesis, synaptic transmission and apoptosis. These combinations of enriched GO-terms were largely in accordance with compound effects reported in literature. Further investigation of the GO-terms for neuron apoptotic process and axon development revealed common genes that were sensitive across different compounds. These genes may therefore be able to serve as general biomarkers for DNT in this assay. On the contrary, the GO-term synaptic signalling, whilst responding to most of the compounds tested, showed little overlap between the conditions as to gene expression patterns within the GO-term. Therefore, this GO term articulates compound-specific effects that may be relevant for revealing differences in neurodevelopmental action, rather than general toxicity. Overall, this evidence that hNPT is a suitable model to detect effects of DNT compounds on key processes in brain development and their underlying mechanism.
the interaction between neuromelanin and neurotoxicants. Furthermore, the dynamic changes in catecholamine levels throughout gray tree frog development may be a target of toxicant-mediated disruption in neurotransmission. These results suggest gray tree frogs may be a promising sentinel species and laboratory model of developmental neurotoxicity and neurodegeneration.

There is a need to efficiently and credibly evaluate developmental neurotoxicity (DNT) hazards. Traditional DNT guideline studies require in vivo rodent models, are inefficient and do not provide mechanistic data. As such, there is an impetus to develop alternative assays for DNT hazard assessment. Here, we have adapted an imaging-based high-throughput phenotypic profiling (HTPP) assay to evaluate developmental neurotoxicity in the hNP1 human neural progenitor cell line. HTPP is a fluorescence-based assay that quantitatively measures changes in cellular organelle morphology. The HTPP assay necessitates cell seeding in 384-well plate format, however, hNP1 cultures have yet to be configured for this format in our laboratory. These cells require pre-coating of the cell growth surface and are typically cultured in antibiotic-free conditions, a potential liability for high-throughput applications where inspection of thousands of assay wells for contamination is not feasible. As such, optimization of three laboratory procedures was needed prior to chemical screening: (1) cell surface coating; (2) cell seeding density in 384-well plates; and (3) cell growth and maintenance protocols that prevent contamination. Each of these processes utilizes a combination of laboratory automation and microfluidics to promote reproducible and efficient preparation of test cultures. The standard cell surface coating procedure for the hNP1 cells requires poly-L-ornithine (PLO; 10 µg/mL) and laminin (20 µg/mL) pre-coat. We optimized the time and temperature for this pre-coat and found that PLO at 4°C up to 96 h and a laminin spike in the cell culture suspension resulted in uniform hNP1 monolayers. Next, we evaluated several hNP1 seeding densities to identify one (10,000 cells/well) that would result in ~60% confluency at the end of the anticipated 48 h assay window, allowing 24 h for cell attachment and growth to 24 h for chemical exposure. Finally, we assessed the effects of antibiotics on cell growth and observed no significant effect on cell number (p = 0.305) or confluency (p = 0.753). Thus, antibiotics will be included in the media for subsequent experiments. The hNP1 culture conditions are now optimized for the HTPP assay, and next we will assess a set of reference chemicals which will be used as assay controls for large-scale chemical screens. This abstract does not reflect US EPA policy.

Characterization of the Gray Tree Frog (Hyla versicolor) as a Potential Model for Developmental Neurotoxicity


Neuromelanin is a pigmented product of catecholamine metabolism that is produced by dopaminergic and noradrenergic neurons in humans and primates. However, neuromelanin is virtually undetectable in rodents. Neuromelanin-containing neurons selectively degenerate in Parkinson’s disease (PD) and in neurotoxicant-primate models. Neuromelanin has been shown to bind and increase cellular levels of PD-relevant toxicants. Thus, the absence of neuromelanin in rodent models is a significant translational weakness. Interestingly, many species of frogs, such as the northern leopard frog (Rana pipiens), form neuromelanin in the brain. Frogs are commonly used as a sentinel species for ecotoxicology and are sensitive to water pollution. Previously, we demonstrated that developmental exposure to perfluoroalkyl substances in northern leopard frogs decreased dopamine in a dose-dependent manner. Here, we aimed to test the hypothesis that the neurobiology of another Indiana-native frog species, the gray tree frog (Hyla versicolor) would be relevant for developmental neurotoxicity studies. This project aimed to (1) quantify catecholamine and neuromelanin content during development and (2) characterize brain histology and neuromelanin content of the gray tree frog. Gray tree frogs were wild-captured and preserved in 4% paraformaldehyde or the brain was removed and flash-frozen in liquid nitrogen at Gosner stages (GS)27-28 (start of tadpole larval stage), GS31-32 (toes begin to separate), GS38-39 (late tadpole stage), GS42 (metaphase start), and GS46 (metaphase completed) (n=13-16/stage). Results demonstrated dopamine, serotonin, S-HIAA, and norepinephrine increase from GS27-28 to GS46. Dopamine levels doubled to 410 pg/mg protein at GS38-39 and were maintained at 330 pg/mg protein by GS46. Serotonin was present at the greatest quantity compared to the other catecholamines, reaching 1338 pg/mg protein by GS46, a six-fold increase from GS27-28. We evaluated gray tree frog brains using dark field imaging for unstained and H&E-stained sections. Preliminary imaging suggests gray tree frog brains contain neuromelanin during GS42. Neuromelanin in these brains presents an opportunity to study the interaction between neuromelanin and neurotoxicants. Furthermore, the

Prenatal Citalopram Exposure Promotes Resilience in Offspring Exposed to Maternal Stress

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Untreated maternal mood and anxiety disorders can have adverse effects on offspring. Nonetheless, it remains unclear how the use of selective serotonin reuptake inhibitors (SSRIs) during pregnancy, while beneficial to mothers, affects children throughout development and during adulthood. Using mice as a model organism, we implemented a chronic unpredictable stress (CUS) paradigm to induce a depressive and anxiety-like phenotype in dams during the mid-latter part of pregnancy. Neurochemical analysis was performed on the brains of offspring at postnatal days 7,14, and 21 using high-performance liquid chromatography (HPLC) and protein concentration analysis. We uncovered a temporally specific alteration in early postnatal serotonin neurochemistry in offspring born to mothers experiencing antenatal stress that was mitigated by maternal citalopram. Furthermore, pups born to stressed dams had elevated protein concentrations in the forebrain at postnatal day 7. Offspring matured until 3 months of age were assessed for changes in behavior through the use of four tests: elevated plus maze (EPM), open field test (OFT), forced swim test (FST), and novelty suppressed feeding (NSF). We found that male pups born to stressed dams exhibited elevated anxiety- and depressive-like behavior. Behavioral alterations were not observed in female mice, suggesting that male offspring are more susceptible to maternal stress. Similar to the neurochemical findings, maternal treatment with citalopram during pregnancy attenuated stress-associated behavioral effects in adult male offspring. Our findings suggest that SSRI treatment during pregnancy in mothers with affective and anxiety disorders may promote resilience in male offspring. Supported by R01MH106806.

Induced Pluripotent Stem Cell-Derived Neural Organoids Generated on Synthetic Hydrogels for Interrogation of Neural Inflammation and Toxicity

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There is a critical need to develop advanced neural models for drug discovery and toxicology applications. Most current in vitro models do not accurately reflect the complexity of neural cell types and important cell-cell interactions and animal models fail to recapitulate the human condition. We have used StemPharm proprietary synthetic hydrogels to develop complex neural organoids generated from iPSC-derived progenitor and differentiated cells in a highly reproducible manner in 96-well plates. Organoids are formed and ready for screening within one month. The organoids are cell-type diverse and RNA-seq analysis demonstrates high intraclss correlation and low coefficients of variation between biological replicates. Importantly, we demonstrated incorporation of microglia into the organoids and their activation as a model of neural inflammation utilizing stimulation with Lipopolysaccharides or Interferon gamma to direct the microglia to a proinflammatory phenotype or with TGFβ and IL-10 or IL-4 and IL-13 to direct microglia to an anti-inflammatory phenotype. Organoids were subject to a screen of known neurotoxins and transcriptional profiles between control and treatment groups were analyzed. We tested 1) chlorpyrifos, an organophosphate insecticide, 2) Lead acetate, known to cause developmental defects, 3) Valproate (valproic acid, VPA), an anti-convulsant and 4) PLX-3397 a CSF-R inhibitor which has been shown to be toxic to microglia. Differential Expression (DE) analysis between treatment groups demonstrated statistically significant changes and these datasets were utilized to identify gene ontology (GO) sets affected by compound treatment and to compare the data to publicly available data sets. We detected perturbations in expected pathways including neural development, axon guidance and mitochondria upon exposure to chlorpyrifos, and depletion of microglia with PLX-3397 treatment. Lead treatment resulted in expected upregulation of GO sets representing response to metal ion and toxin as well as metalloprotein production and decreases in extracellular matrix genes. Both Lead and VPA treatment also resulted in the downregulation of GO sets involved in inflammatory and innate immune responses and microglial-specific genes demonstrating the importance of using multicellular
Developmental neurotoxicity (DNT) represents a critical hazard for which less than 1% of environmental chemicals have been screened. Despite this backlog, in vivo guideline studies of DNT are resource intensive, low throughput, and yield little insight into the molecular mechanisms underlying chemical-induced DNT. To compound matters, batteries of in vitro assays have been developed to elucidate the phenotypic and mechanistic underpinnings of DNT attributed to environmental chemical exposure. Recently, we utilized a screening approach using rat cortical neurons grown on microelectrode arrays (MEA) to assess the effects of chemical compounds on neural network formation. These assays collect and characterize multiple neural network parameters including neuronal firing rates, burst frequency/character, and coordinated network activity, alongside common measures of cell viability and morphology. Here we have coupled these data to high-throughput transcriptomics to resolve molecular key events mediating disruption of neural circuit formation. Using data from the MEA assay, we have identified “tipping point” concentrations at which chemical exposures elicit irreversible divergences from normal cortical circuit development. Specifically, we examined 18 chemicals in the classes of organophosphates, metals, and GABA modulators and report cognate changes in gene expression and associated cellular pathways. Gene Ontology analysis reveals that across classes at tipping point concentrations, there are unique transcriptional perturbations in gene programs for Neuronal Development, Neuron Migration, and Axonogenesis, among others. Despite this overlap, we see very distinct sets of genes affected by each chemical and class individually, suggesting that a characterization of the molecular events occurring in each cortical cell type will be key to understanding the complex interplay of transcriptional and circuit plasticities affected by these compounds. As such, we are currently generating a combination of our MEA assay with single-cell sequencing data in order to identify key molecular events with cell-type resolution. These data will aid in developing putative adverse outcome pathways by linking key molecular events associated with altered neural network development. This abstract does not necessarily reflect US EPA policy.

Establishment of 3D Neurosphere Culture from Human iPSC-Derived Neural Progenitor Cells

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Human induced pluripotent stem cells (iPSCs)-derived neural progenitors provide an advanced in vitro system for modeling the human brain. This system can be used to investigate brain development, disease pathology, toxic effects of environmental chemicals, or perform drug screening. Here we use iPSC-derived neural progenitor cells (NPCs) to generate 3D neurospheres in vitro. We describe a straightforward method of generating neurospheres and describe their utilization for experimental applications such as dopaminergic differentiation and drug toxicity assays. We found that NPC-derived neurospheres grew exponentially and maintained their progenitor state for up to two weeks in culture. Further we found that neurospheres were able to successfully differentiate to multiple brain lineage cells including dopaminergic neurons in 3D. These spheres displayed higher tyrosine hydroxylase (TH)-positive cells compared to NPCs in 2D cultures. Neurospheres from normal donor cells successfully differentiated and expressed the TH marker uniformly. However, neurospheres from Parkinson’s disease donor cells displayed different patterning post differentiation than the neurospheres from normal donor cells. Neurospheres treated with various chemotherapeutic agents in multiple doses gave differential responses between healthy and diseased cells. Viability of healthy neurospheres were significantly affected by paclitaxel and vincristine at all three dosages as compared to the control whereas no significant differences were seen for neurospheres from disease donor cells. Similar difference in sensitivity to paclitaxel and vincristine was observed in the two different NPCs. Interestingly, NPCs from healthy donors demonstrated greater sensitivity to paclitaxel and vincristine as compared to the neurospheres derived from the same cells. This trend was also seen for NPCs and neurospheres from the diseased donor. Responses to amiodarone and chlorhexidine were found to be similar between both neurospheres from healthy and diseased donors. However, NPCs were slightly more sensitive to amiodarone and chlorhexidine as compared to their neurosphere counterparts. These data demonstrate that iPSCS-derived neurospheres are a powerful tool for developmental studies, drug screening, and toxicity testing compared to 2D NPC cultures.

Developmental Deltamethrin Exposure Alters Neurotransmitters, Long-Term Potentiation, Cytokines, and Apoptosis in Sprague-Dawley Rats

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Pyrethroids are a prevalent class of insecticides that act through voltage gated sodium channels to facilitate long channel opening and delay closings. Pyrethroids are widely used in areas where children are present. Limited epidemiological data suggest that children exposed to pyrethroids have neurobehavioral abnormalities. The effects of Type II pyrethroids, such as deltamethrin (DLM), on development have received little attention. We exposed rats to DLM from postnatal day (P) 3-20 and found deficits in egocentric and allocentric learning and memory primarily in males. Here we report on Sprague-Dawley rats treated with 0 or 1.0 mg/kg/day DLM as before. Microdialysis revealed decreased amphetamine stimulated dopamine release in the nucleus accumbens of DLM-treated rats, with no change in potassium stimulated glutamate release in the hippocampus. Western blots revealed that NMDA receptor subunits were altered in male DLM-treated rats, but not in females. No change in dopamine receptors were seen by western analysis. Preliminary data indicate that inflammatory cytokines are also altered by developmental DLM-treatment measured on P20. Cell death was increased at P20 in DLM-treated rats in striatum and hippocampus as seen by TUNEL staining. Hippocampal CA1 LTP was increased in DLM-treated rats 1-2 weeks after exposure (P28-35) in males and females, but only in males as adults. These effects raise concerns about the safety of DLM from developmental exposure and merit closer scrutiny in future experiments. Supported by NIH training grant T32 ES007051.
Cadmium (Cd) is a ubiquitous toxic heavy metal of major public concern. Despite inefficient placental transfer, maternal Cd exposure impairs fetal growth and development. Increasing evidence from animal models and humans suggests maternal Cd exposure negatively impacts neurodevelopment; however, the underlying molecular mechanisms are unclear. To address this, we utilized multiple -omics approaches in a mouse model of maternal Cd exposure to identify pathways modulated in the developing brain. Offspring maternally exposed to Cd presented with enlarged brains proportional to body weights at birth and altered behavior at adulthood. RNA-seq identified increased Hox gene and myelin marker expression and suggested alterations to retinoic acid (RA) signaling in maternally-exposed newborn brains. Proteomic analysis showed altered levels of proteins involved in cellular energy pathways, hypoxic response, and RA signaling. Consistent with transcriptomic and proteomic analyses, we identified increased levels of retinoids in maternally-exposed newborn brains. Metabolomic analyses identified three metabolites with altered abundance, consistent with changes to cellular energy pathways and hypoxia. Finally, maternal Cd exposure reduced mitochondrial DNA levels in newborn brains. The identification of multiple pathways perturbed in the developing brain provides a basis for future studies determining the causative links between maternal Cd exposure and altered neurodevelopment and behavior.

Benzo[a]pyrene (BaP) is a pollutant and a known carcinogen. Exposure from BaP can come from various sources such as vehicle emissions, tobacco smoke, and grilled food. BaP exposure has been linked to delays in neurological development in both animals and humans. We used a mouse model to mimic the human genetic variation in genes related to BaP metabolism, the aryl hydrocarbon receptor (AhR), to model human genetic variation. Pregnant dams were treated with 10mg/kg/day BaP in corn oil-soaked cereal or the corn oil vehicle from gestational day 10 to postnatal day 25. One male and one female per litter were assigned to behavioral testing. Following behavioral testing at postnatal day 120, striatum, hippocampus, prefrontal cortex, and hypothalamus were collected. Dopamine, serotonin and their metabolites were measured using High-Performance Liquid Chromatography with Electrochemical Detection. There was a significant gene x treatment interaction in the hippocampus with BaP-treated wild type Ahr/Cyp1a2(+/+) and poor affinity AhrdCyp1a2(-/-) mice having lower dopamine and DOPAC levels compared with corn oil-treated controls (P < 0.01). In contrast, BaP-treated high affinity Ahr/Cyp1a2(+/+) showed the greatest changes in the prefrontal cortex with significantly lower serotonin levels (P < 0.05) and a trend for decreased DOPAC (P = 0.89). Together, these findings suggest that all three genotypes show some susceptibility to developmental BaP exposure.

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Benz[a]pyrene (BaP) is a carcinogenic polycyclic aromatic hydrocarbon commonly found in traffic-related air pollution, tobacco smoke, and grilled foods. BaP is linked to learning deficits and to neurodevelopmental delays in human and animal studies. We are using a mouse model to determine if genetic differences increase susceptibility to BaP exposure during early brain development. Mice with variations in the aryl hydrocarbon receptor, lacking the CYP1A2 metabolic enzyme and wild type control mice were exposed to 10mg/kg/day BaP from gestational day 10 (GD10) through weaning at postnatal day 25 (P25). One male and one female per litter were randomly selected for neurobehavioral testing. A battery of cognitive and motor function tests were performed when the mice reached early adulthood (P60). We used Novel Object Recognition and Morris Water Maze to assess hippocampal dependent non-spatial and spatial learning and memory. BaP-treated knockout mice spent less time exploring the novel object, but the differences did not quite reach statistical significance (P = 0.51). BaP-treated AhraCyp1a2(-/-) mice had significantly longer path lengths on Days 3, 5 and 6 in the Acquisition Phase of Morris Water Maze (P < 0.05). BaP-treated mice had significantly impaired reference memory in the Acquisition and Shift-reduced Probe trials (P < 0.05). We will test additional cohorts until we have reached n ≥ 16 litters per group.

Habituation represents a non-associative form of learning that is conserved from unicellular organisms to invertebrates and humans. It is characterized by iterative response decrements to repeated inconsequential stimuli in order to reduce unnecessary physiological cost. Impairment of habituation is prevalent in human disorders including autism, schizophrenia, attention deficit hyperactivity disorder, and drug addiction. In addition to genetic predisposition, exposure to environmental factors, including xenobiotics, is linked with adverse neurodevelopmental outcomes. However, there is a distinct lack of automated screening assays to detect the effects of developmental exposure to environmentally relevant chemicals on learning- and memory-related outcomes. Teleost fish, including zebrafish, exhibit a rapid escape motor behavior termed the acoustic startle response (ASR). Upon repeated acoustic stimulation, larvae undergo habituation such that the organism learns not to respond to the non-threatening stimulus. While acute pharmacological modulation of ASR by serotonergic, glutamatergic, and dopaminergic agents has been demonstrated in zebrafish, little is known regarding the effect of developmental exposure to environmental chemicals on learning and memory formation, nor has habituation been tested in parallel with other more commonly used automated behavior assays. Therefore, we established a sequential, automated zebrafish behavior-based pipeline to evaluate locomotor responses to light stimuli in addition to acoustic sensitivity, habituation, and recovery. The assay was systematically optimized for age, stimuli frequency, acclimation requirements, and inter-test, inter-bout, and inter-stimulus intervals. We report that larval zebrafish at 5 but not 4 days post-fertilization demonstrate robust habituation behavior and that a 6 min inter-test interval is required to achieve ASR recovery. In line with previous pharmacological studies, acute exposure to the agonist NMMA (1.4 µM) and the NMMA antagonist ketamine (1 mM) reduced habituation learning, demonstrating assay functionality. Together, the assay will ultimately bridge a gap between environmental and human neurotoxicology via screening xenobiotics for developmental disruption of an evolutionarily conserved mechanism of habituation learning.

Neural stem cells (NSCs) play an essential role in shaping the developing brain. Recently, it’s been proposed that placenta plays a critical role in fetal neuroprogramming through the inter-relationships between neurotoxins and the placenta. Placental cues can reach the fetal brain through the immature blood-brain barrier (BBB) inducing physiological or pathological changes with consequent neurodevelopmental outcomes. Placental trophoblasts actively release nano-sized membrane-bound extracellular vesicles (EVs). The EVs can cross the fetal BBB and shuttle cargoes of bioactive molecules, such as proteins, lipid, and nucleic acids. We hypothesize that placental EVs modify NSC functions by transferring cargoes of bioactive molecules to NSC. We further hypothesize that environmental metal exposure such as arsenic (As) in utero alters fetal brain development by disrupting normal cargoes of placental EVs. To test these hypotheses, we first harvested EVs from the culture medium of a human first trimester placenta cell line HTR-8/SVneo using ultracentrifugation. Then, ReNcell Cx human neuroprogenitor cells were incubated with the harvested EVs in the absence or presence of As. We found treatment of placental EVs stimulated proliferation of ReNcell Cx cells. We further showed that pretreatment of ReNcell Cx cells with placental EVs protects against As-induced inhibition of cell proliferation. Proteomics analysis revealed that brain-enriched proteins have been detected in placental EVs. Our study proposes placental EVs as a novel signaling factor mediating the NSC responses to As exposure thereby linking environmental exposures to health trajectories regulated by the placenta. Further study will be warranted to elucidate the mechanisms by which placental EVs affect NSC proliferation against As exposure.

Thyroid hormones (TH) are essential for brain development, and their synthesis is dependent on thyroxoperoxidase activity in the thyroid follicles. The U.S. Environmental Protection Agency’s ToxCast program has identified a number of environmental chemicals that are thyroxoperoxidase inhibitors in vitro and therefore potential thyroid hormone system disruptors. However, what effects these chemicals can have on in vivo serum TH concentrations or on brain development remain unknown. Here, we exposed pregnant rat dams by oral gavage to 2-mercaptobenzimidazole (MBI), amitrole or cyanamide; three environmental chemicals that are in vitro inhibitors of thyroxoperoxidase. MBI and cyanamide had only minor, sporadic effects on TH concentrations in dams but caused reproductive toxicity such as dystocia and reduced postnatal body weights. In contrast, amitrole, a triazole pesticide, reduced circulating thyroid hormone (T4) concentrations and caused abnormal brain development in the offspring. A congenital malformation were observed in the brain of the offspring, with large clusters of ectopic neurons in the corpus callosum; so called periventricular heterotopia, which can form after developmental TH insufficiency. Since the irreversibly formed heterotopia appears to manifest after exposure to amitrole, this pesticide joins the two classical thyroperoxidase inhibiting anti-thyroid drugs propylthiouracil and methimazole. We propose heterotopia formation as a relevant effect endpoint for developmental thyroid hormone system disruption caused by exposure to thyroxoperoxidase inhibiting chemicals.
2452 Bypassing the Brain Barriers: Identification of Serum microRNAs Reflective of Developmental Neurotoxicity

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Evaluating the neurodevelopmental effects of thyroid disrupting chemicals like flame retardants, perfluorinated compounds, and pesticides is challenging. While current regulatory tests attempt to determine if these chemicals may affect the developing brain, existing strategies are expensive, time consuming, and lack sensitivity. Previously we characterized that transient developmental thyroid hormone (TH) insufficiency alters cell adhesion and cell survival at the ventricular epithelium in neonatal rats. As this progenitor cell population functions as one of the protective brain barriers (cerebrospinal fluid-idbrain barrier), we hypothesized that these abnormalities may permit “leaking” of small molecules from the brain tissue and back into the circulation. These small molecules could then be identified in blood samples, serving as a direct readout of abnormal neurodevelopment. To address this hypothesis pregnant rats were treated with a low dose of propylthiouracil (3 ppm) via the drinking water to induce TH insufficiency beginning on gestational day 6, and dams were permitted to give birth. This treatment reduced THs in both the serum and brain of neonates relative to controls during the postnatal period. Next, we performed small RNA sequencing (RNA-Seq) of sera and brain tissue (telencephalon) in neonates on postnatal day 8 to identify small non-coding RNAs that may reflect the observed cellular abnormalities at the ventricle. Of the differentially expressed RNAs identified, seven microRNAs were upregulated in the serum of hypothyroid pups as compared to controls (FDR corrected p-values <0.05). Interestingly, these microRNAs have been linked to neuronal apoptosis and endothelial dysfunction in the brain, directly paralleling abnormalities we identified within the brain tissue of littermates. These data show that serum microRNAs may be a novel tool to detect and monitor developmental neurotoxicity by a rapid, non-invasive method in regulatory studies. Additionally, as the microRNAs identified are conserved in humans, we are considering the utility of these biomarkers to monitor the health effects of TDCs in children. This work does not reflect US EPA policy.

2453 Thyroid Hormone Action Controls Cell Signaling in the Developing Ventricular Epithelium: Implications for a Mechanistic Adverse Outcome Pathway

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Developmental thyroid hormone (TH) insufficiency is associated with various neurodevelopmental disorders in children. Previously, we demonstrated that developmental thyroid hormone insufficiency alters cell adhesion and apoptosis in the neonatal rat brain. These abnormalities were largely localized to the ventricular epithelium, a progenitor cell niche, and later resulted in a periventricular heterotopia. This permanent morphological abnormality now serves as an apical endpoint in a putative adverse outcome pathway (AOP). To further elucidate this AOP we employed laser capture microdissection and RNA-sequencing (RNA-Seq) to evaluate how reduced thyroid action may affect this cell population and result in periventricular heterotopia. Pregnant rats were treated with propylthiouracil (PTU, 0.003%) through the drinking water to induce maternal TH insufficiency from gestational day 6 until postnatal day 14 (PN14). This goitrogen treatment significantly reduced total thyroxine (T4) but not triiodothyronine (T3) in the sera of dams, and both T4 and T3 were significantly reduced in the pups during the postnatal period. Both T4 and T3 were also significantly reduced in the telencephalon of exposed neonates on PN2 relative to controls. Next, frozen sections were collected from pup brains on PN2; this developmental stage precedes heterotopia formation and is a known window of susceptibility. The posterior ventricular epithelium was microdissected, and RNA sequenced using Illumina HiSeq. We identified 268 differentially expressed genes in the ventricular epithelium of PTU-exposed animals compared to controls (adj. p-values <0.05). Using Ingenuity Pathway Analysis, we show that many of these genes are associated with cell junction maintenance, congruent with our previous study. These results support the hypothesis that differential expression of genes involved in cell junction structure and function may serve as crucial key event(s) of heterotopia formation, and these data increase the biological plausibility of our putative AOP. This work does not reflect US EPA policy.
contaminates drinking water sources in agricultural regions where this herbicide is applied to crop fields. This study tested the central hypothesis that an early developmental exposure to Pb and ATZ mixtures results in a greater than additive mixture toxicity response. The zebrafish model system was applied to test the central hypothesis with exposures to 3 ppb (µg/L) ATZ, 30 ppb ATZ, 100 ppb Pb, or mixtures of 3 ppb ATZ with 100 ppb Pb or 30 ppb ATZ with 100 ppb Pb beginning at 1.5 hpf and continuing through 120 hpf. Following the exposure behavioral alterations were assessed in larval using the Noldus DanioVision white light routine. Locomotor data was analyzed with an analysis of variance (ANOVA) and post-hoc least significant difference test when appropriate (α=0.05). Behavior data showed hyperactivity in the 3 ppb ATZ treatment group for total distance moved, mean velocity, and time spent moving (p<0.05). In addition, the two 100 ppb Pb mixtures with either 3 ppb ATZ or 30 ppb ATZ also showed hyperactivity for total distance moved, mean velocity, and time spent moving (p<0.05), while no significant behavioral changes were observed in the 100 ppb Pb or 30 ppb ATZ treatments alone (p>0.05). These results support a greater than additive mixture toxicity response that warrants further investigation.

**Human Induced Pluripotent Stem Cell-Derived Neural Platforms Rapidly Assess Toxicity of Potential Antiviral Therapeutics**


Given the speed with which viral threats can rise to pandemic levels, there is an urgent need for antiviral therapies to combat them. Repurposing existing medications is a way to reduce both the time and cost of drug development. However, because these infections are non-discriminatory and medications may be applied to new population segments, sophisticated screening systems are needed to ensure safety across new patient demographics. Here, we present human iPSC-derived cell-based neural screening platforms to investigate the toxicological profile of potential therapeutic compounds acting at different stages of neural development to fully understand the neurotoxicity of VPA exposure using zebrafish as a model or different stages of neuronal development to fully understand the neurotoxicity profile of potential therapeutics across normal ontogeny.

**Folic Acid Supplementation Rescues Valproic Acid-Induced Developmental Neurotoxicity and Behavioral Alterations in Zebrafish Embryos**

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Fetal exposure to the anticonvulsant drug valproic acid (VPA), used to treat certain types of epilepsy, increases the risk for birth defects, including neural tube defects, as well as learning difficulties and behavioral problems. Here, we investigated neurotoxic effects of VPA exposure using zebrafish as a model organism. We exposed zebrafish embryos to valproic acid (FA; 100 µM) during gastrulation to rescue the VPA-induced neural and behavioral perturbations was also examined. Zebrafish embryos of different transgenic lines with neuronal GFP expression were exposed to increasing concentrations of VPA with or without FA supplementation. Fluorescence microscopy was used to visualize alterations in brain structures, neural progenitor cells, as well as motor neurons and neurite sprouting. A locomotor behavioral assay was used to examine the functional consequences of VPA and FA treatment in wild type zebrafish embryos. In zebrafish embryos, VPA exposure caused a decrease in the midbrain size, an increase in the midline gap of the hindbrain, and perturbed neurite sprouting of secondary motor neurons, in a concentration dependent manner. VPA exposure also decreased the fluorescence intensity of neuronal progenitor cells in early developmental stages, indicating fewer cells. Furthermore, VPA exposure significantly altered embryonic twitching activity causing hyperactivity in dark and hypoactivity in light. Supplementation of FA rescued the VPA-induced smaller midbrain size, and hindbrain midline gap defects. FA treatment also increased the number of neuronal progenitor cells in VPA-treated embryos and salvaged neurite sprouting of the secondary motor neurons. FA rescued the VPA-induced alterations in locomotor activity in light, but not in dark. We conclude that VPA exposure induces specific neurotoxic perturbations in developing zebrafish embryos, and that FA reversed most of the identified defects. The results demonstrate that zebrafish is a promising model to study VPA-induced teratogenesis and to screen for countermeasures.

**Metabolite Reprogramming by In Utero Maternal Benzene Exposure**

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Environmental chemicals play a significant role in the development of metabolic disorders, especially when exposure occurs early in life. We have recently demonstrated that benzene exposure, at concentrations relevant to cigarette smoke, induces a severe metabolic imbalance in a sex-specific manner affecting male but not female mice. However, the roles of benzene in the development of aberrant metabolic outcomes following gestational exposure, remain largely unexplored. In this study, we exposed pregnant C57BL/6JB dams to benzene at 50 ppm or filtered air for 5 days/week (6h/day from gestational day 1 to birth) and studied male and female offspring metabolic phenotypes in their adult life. While no changes in body weight or body composition were observed between groups, 4-month-old male and female offspring exhibited reduced parameters of energy homeostasis (VO2, VCO2, and heat production). However, only male offspring from benzene-exposed dams were glucose intolerant and insulin resistant at this age. By six months of age, both male and female offspring displayed glucose and insulin intolerance, associated with elevated expression of hepatic gluconeogenesis and inflammatory genes. Additionally, this effect was accompanied by elevated insulin secretion and increased beta-cell mass only in male offspring. Thus, gestational benzene exposure can reprogram offspring for increased susceptibility to the metabolic imbalance in adulthood with differential sensitivity between sexes.

**Methylmercury Alters Relative Abundance of Cell Types in In Vitro Human Cortical Neuronal Development and Exhibits Persistent Effects on Neuronal Function**

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We assessed the effects of methylmercury (MeHg) on the development of cortical glutamatergic neurons differentiated from human induced pluripotent cells (hiPSCs). We exposed differentiating cultures either during the neural precursor stage (NP, days 4-10) or during the NP and intermediate progenitor cell stage (IP, NP+IP; days 4-10 and 14-20) to 0.1 or 1 µM MeHg. Neither of these two exposure paradigms resulted in loss of neuronal viability. The differentiation was then continued in the absence of MeHg, the cells were harvested on day 38 and subjected to single cell RNA sequencing using the 10x Genomics platform. Based on gene expression analysis we identified nine distinct cell clusters: radial glial cells (clusters 0 and 1) representing the majority of cells (~63%), intermediate progenitor cells (cluster 5, ~5%), postmitotic immature VGLUT1 glutamatergic cortical neurons (cluster 2, ~18%) and other forebrain immature VGLUT2 glutamatergic neurons (cluster 3, ~8%), anterior telencephalon choroid plexus and astrocyte-like progenitors (cluster 4, ~4%) and clusters 6, 7 and 8 predicted to be other radial glial and mugal cell types collectively representing ~2% of remaining cells. We observed no novel or missing clusters resulting from MeHg exposure, but we did observe significant changes in the fraction of cells in some clusters. Thus, MeHg exposure (1 µM) during the NP stage significantly decreased the fraction of cluster 3 cells by 40%, while the longer NP+IP exposure caused a smaller 19% loss. Progenitor cells (clusters 0 and 1) were generally less sensitive, and we observed a small, but significant decrease in radial glial cluster 1 (13%) in 1 µM NP+IP treated cultures, and a significant 12% increase in radial glial cluster 0 cells after 1 µM NP treatment. The choroid plexus progenitor cells appeared the most sensitive with NP- and NP+IP treatments resulting in significant 59.1% and 291% increases, respectively. Thus, not only are cell clusters affected differentially, but the timing of treatment is also important for the outcome of developmental MeHg exposure. MEA analysis at day 64-67 of differentiation revealed a 22- and 45-fold increase in neural activity in cultures treated with 1 µM MeHg during the NP- and NP-stage, respectively, further emphasizing the importance of the timing of MeHg exposure and demonstrating that developmental exposures can result in long-lasting functional consequences. NIEHS R01 ES07331, ABB, MA.
while thyroid disease has a complex etiology, exposure to endocrine disrupting chemicals (EDCs) is correlated with reduced serum thyroxine (T4) in some populations. As thyroid hormones (THs) control brain development in children, determining the health effects of EDCs is paramount. However, evaluating the neurodevelopmental effects of such chemicals in animal models is challenging as it is unclear how reduced serum T4 may correlate to TH action within the brain. To address these limitations, we hypothesized that differential gene expression could serve as a biomarker of hormone action, which could strengthen interpretations of serum T4 measures. While we previously investigated mRNA expression, here we performed small RNA-sequencing (miRNA-Seq) at a critical developmental stage in the rat to evaluate small non-coding RNA (RNA) expression.

These molecules often control transcription of multiple genes simultaneously and can be highly informative of cellular dynamics. To investigate if TH action controls small RNA expression in the brain, pregnant rats were administered the goitrin polyphenolicurea (0 or 3 ppm) in their drinking water from gestational day 6 until postnatal day 14 (PN14). This exposure reduced dam T4 by ~50% while triiodothyronine (T3) was comparable to controls, suggesting that the thyroid axis was not severely affected on PN14. In contrast, pup serum T4 and T3 were both significantly decreased on PN8 (~94% and ~45%, respectively) as was brain T4/T3 (~94% and ~59%). Next, miRNA-Seq was performed on the PN8 pup forebrain, which identified differentially expressed microRNA (miR) (p-value < 0.05). Gene ontology analyses revealed that Wnt signaling, neuronal differentiation, and cell adhesion are pathways targeted by these microRNAs. To support these data, immunofluorescence of littermates demonstrated that cell adhesion was abnormal in TH insufficient pups. Together, these results show that reduced maternal serum T4, even in the absence of reduced T3, is associated with differentially expressed microRNAs and altered cytoarchitecture in the pup brain. Given the stability of microRNAs in tissues, it is possible that targeted assessment of these molecules could serve as a readout of TH action in development and reproductive toxicity studies. This work does not necessarily reflect US EPA policy.

2646a Reduced Maternal Thyroxine Alters microRNA Expression and Cytoarchitecture in the Developing Rat Brain
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2646 Structural, Cellular, and Molecular Neurotoxicity of Chronic, Low-Level Exposure to the Marine Toxin, Domoic Acid in Nonhuman Primates
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Domoic acid (DA), a glutamate agonist, is produced by marine algae and can bioconcentrate in shellfish. Current regulatory limits constrain human exposure to 20 ppm in shellfish (~0.075-0.1 mg/kg), but changing environmental conditions are leading to more frequent long-term high DA blooms. To understand how low-level, chronic exposure to this toxin impacts the limbic system of the brain, the present study used targeted magnetic resonance imaging (MRI) in the fornix, fimbria, and internal capsule, histopathology in the hippocampus and thalamus, and hippocampal gene expression studies to assess neurotoxicity of DA in a nonhuman primate model. Twenty-eight adult, female Macaca fascicularis were orally exposed to 0, 0.075, 0.15 mg/kg/day for up to two years. A subset of these females (n=12) underwent a single, sedated MRI scan in vivo, to assess volumetric and tractography changes in the hippocampus, thalamus, and connecting white matter tracts, all animals were necropsied to evaluate the cellularity and morphology of the neurons, extracellular matrix, and microglia in these regions. Real-time PCR analysis of the hippocampus was conducted on 11 of the animals. MRI and histopathology evaluations did not suggest signs of overt neurotoxicity, but revealed that some animals, especially in the 0.15 mg/kg/day DA exposure group, expressed focal microglia reactions within both white and gray matter structures of the limbic system. Of the 2544 differentially expressed genes in the hippocampus revealed that key pathways in neurological function, including those in opioid and calcium signaling, were also affected (p<0.0001). These results suggest that chronic exposure to DA near the human regulatory limit does not lead to acute neuropathic effects but may induce microglial responses and alter neurotoxicity. This work does not necessarily reflect US EPA policy.

2647 Neurite Retraction of Monoaminergic Neurons following Exposure to Conditioned Medium of Acrylamide-Treated Microglia and Transcriptomic Analysis
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Neurotoxicity of environmental electrophiles such as 1-bromopropanone (1- BP) or acrylamide (ACR) has been reported in humans and experimental animals, while the underlying mechanism remains obscure. Our previous studies demonstrated that exposure to 1- BP or ACR causes degeneration of retinal ganglion cells in mice. In this study, 1C11 mouse retinal stem cell line and BV2 microglia cell line were used to investigate the mechanisms of ACR-induced degeneration of monoaminergic neurons. 1C11 stem cells were differentiated into noradrenergic neural cells (1C11 NE) and 5-HT neurons. Further analysis of RNA-seq data is ongoing to investigate changes of the gene expression profile.
Montelukast (MTK) is widely used for asthma management. This cysteinyl leukotriene receptor (CysLT1) inhibitor is used to prevent and treat asthma symptoms. The results demonstrated that the treatment with SFN resulted in dose-dependent protection against ACR-induced neuropathy. Further studies are needed to clarify the mechanism how SFN attenuates the neurotoxicity of ACR.

The blood-brain barrier (BBB) is a critical interface between the central nervous system and the rest of the body. It regulates the exchange of molecules between the bloodstream and the central nervous system (CNS), preventing harmful substances from entering the brain. To identify a potential thiolomic-wide change.

Numerous studies have shown that oxidative stress plays a crucial role in the development of neurological diseases. The results reported that exogenous acrylamide exposure induced oxidative stress and inflammation, which contributed to the development of ACR-induced neuropathy. Moreover, the results also demonstrated that SFN treatment significantly reduced oxidative stress and inflammation in the brain, suggesting its potential therapeutic effect.

The environmental contaminant, ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy) propanoic acid (GenX), has been reported to induce oxidative stress and inflammation in the brain. The results showed that SFN treatment significantly reduced oxidative stress and inflammation in the brain, suggesting its potential therapeutic effect.

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Drug-induced seizure liability has become a major challenge for the pharmaceutical industry over the last 10 years. The examination of the central nervous system (CNS) in non-rodents is traditionally limited to behavioral observations or histopathological examination. Evidence of tremors, twitches or convulsions could potentially be a potential indication for seizure risk of new drugs. However, these effects can manifest with the absence of convulsions; nevertheless, electroencephalogram (EEG) assessments are generally only applied if a neurological concern is identified. Therefore, often limited information is available regarding potential CNS safety liabilities before advancing a drug candidate to the clinic. We aimed to identify seizure-sensitive liabilities using innovative in-vivo methods which combine EEG and behavioral assessments and measurements of selected neural biomarkers. Female beagle dogs were implanted with a radio-telemetry device for continuous EEG, temperature and activity recordings. This radio-telemetry approach was combined with synchronized video monitoring for continuous observations of the dogs in their home-cage. Two reference compounds were selected for the model validation of observations of convulsions reported in previous studies: an A2A/A2 antagonist and pentylenetetrazol. Four dogs were dosed via oral gavage with an A2A/A2 antagonist compound at 5, 20 and 75 mg/kg, and monitored for 24 hours. None of the animals showed seizurogenic activity at any of the selected doses. At higher doses, behavioral observations included decreased righting, anorexia, tremors and convulsions. EEG spectral analysis revealed important changes in a large range of frequencies (i.e. 0.5-60 Hz) when compared to values prior to PTZ infusion. In conclusion, the EEG telemetry system combined with the video recordings is a useful application to improve seizure risk detection.

2469 Evaluation of Cr(VI) Neurotoxicity and Its Potential as a Brain Gerontogen

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Today our societies are challenged with the health burdens of environmental pollution and a rapidly aging population, with 1 in 5 Americans reaching geriatric age by the year 2050. This prolonged aging combined with environmental exposures, we urgently need to understand how environmental pollution affects an aged body differently from a younger body and how environmental pollution contributes to aging phenotypes. Traditionally, the brain was considered composed of mostly post-mitotic neurons and hence genotoxic agents were considered less of a threat. Now, we know the opposite: true cells of the brain, the glia, are mitotic and play critical roles in protecting and supporting neuronal health. Recent studies show these glial cells can exhibit increased aneuploidy and chromosome instability with aging and early in neurodegenerative diseases. Hence, there is a critical need to understand how genotoxic chemicals affect brain health and contribute to premature aging. Hexavalent chromium (Cr(VI)) is a major environmental health concern that can induce aging phenotypes and has the best defined clastogenic mechanism of metals. Cr(VI) also causes brain damage that may be linked to a variety of neurological symptoms. We propose to investigate the role of Cr(VI) in this aging paradigm, using young vs middle-aged vs geriatric rats exposed to Cr(VI) for 90 days via drinking water. We hypothesize aged individuals are more susceptible to Cr(VI)-induced neurotoxicity and that Cr(VI) induces brain aging by causing aneuploidy and chromosome instability resulting in glial senescence. Here we present preliminary data demonstrating how zinc chromate exposure in rats results in brain metal dyshomeostasis, and Cr(VI) toxicity, to M059K and M059J cells (derived from a glioblastoma, compared to M059K, M059J lacks DNA double strand break repair response). Rats were given an oral aspiration of zinc chromate twice weekly at 0, 0.4, or 0.8 mg Cr/kg for 24 hours or 90 days. ICP-MS analyses of four brain regions revealed that female rats accumulated Zn over the 90 d exposure and exhibited decreased Zn/Fe ratio across brain regions. However, there was no significant increase in brain Cr(VI) level after 90 d exposure. Ongoing work is focused on developing further mechanistic insight into this pathway from Cr(VI)-induced DNA damage to cellular senescence in glia of the brain with the in vivo rat and cultured glioblastoma cell models.

2470 Effects of Acute Hydrogen Sulfide Exposure on the Brainstem

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Hydrogen sulfide (H2S) has a long history of toxicity as well as physiological functions within important systems including cardiovascular, respiratory, and central nervous systems. H2S incidents vary by occupational hazard including oil refineries, intensive confinement, and water treatment plants; however, the lethal properties of the gas are undeniable. The main cause of death after acute H2S exposure is still a mystery. The effects of H2S on the brainstem and respiratory center are important area that have not been investigated. Our hypothesis is that the inhibition of the activity of the acute H2S poisoning are mediated through serotonin (5HT) and GABA receptors. To these questions we exposed male C57BL/6 mice to 1000ppm of H2S for 50min or until 50% mortality and euthanized at 5min, 2h, 12h, 24h, 72h, and 7d post-exposure to evaluate neurodegeneration in a timewise manner. In this study we investigated catalepsy, motor activity, and microtubule formation as well as neurotransmitters as receptors and enzymes involved in respiratory rhythm in the brainstem. Among these we measured dopamine (DA), DOPAC, homovanillonic acid (HVA), serotonin (5HT), 5-hydroxyindoleacetic acid (5-HIAA), norepinephrine (NE), GABA, glutamate, and glycine. Additionally, we analyzed for neurodegeneration in different regions of the brainstem using immunohistochemical staining Iba1, GFAP, and NeuN. Data presented as mean and standard error of the mean. We found that DA and its metabolites, DOPAC and HVA, are increased at 5min; at 2hr HVA remained elevated. NE was significantly decreased at 5min following exposure. There was no change in 5HT or 5HIAA concentration at any timepoint investigated. Among the amino acid neurotransmitters, glutamate decreased starting at 24hr and continued decreasing at 72hr and 7d. Out of all the SHT receptors investigated, SHT3AR concentrations were increased starting at 24hr and remained elevated at 72hr and 7d. Immunohistochemical analysis revealed neurodegeneration begins starting at 24hr and continues through to 7d. The brainstem contains many types of neurons including dopaminergic (DAergic) and glutamatergic neurons that are important for regulating respiratory rhythm. DA and glutamate have been found to play a role in inspiratory rhythm and we believe that glutamatergic neurons are affected during exposure but we do not see any physical changes until neurodegeneration sets in. It is apparent that DA is affected during exposure as demonstrated by increased concentrations of it and its metabolites. In future studies, we would like to confirm which specific neuron pathways are affected by H2S, DAergic or glutamatergic pre-inspiratory or inspiratory neurons. We conclude that H2S targets key regions in the brainstem during exposure which lead to eventual neurodegeneration of the respiratory center.

2471 Circulating HMGB1 Triggers the Persistent Pro-inflammatory Microglia Phenotype: Implications for Gulf War Illness

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Gulf War Illness (GWI) is a chronic, multi-symptom condition that affects approximately 30% of the U.S. veterans who served in the 1990-1991 Gulf War and is characterized by both central nervous system (CNS) and peripheral symptoms. Persistent microglial dysregulation is implicated in GWI, but the role of circulating factors in driving the continuous proinflammatory pathology is poorly understood. HMGB1 is an alarm actively secreted by CNS and peripheral immune cells to function as an autocrine and paracrine pro-inflammatory signal, but the role of circulating HMGB1 in persistent neuroinflammation and GWI remains largely unknown. We began to address these issues using an LPS model of the persistent microglial pro-inflammatory response, where male C57BL/6 mice injected with LPS (5 mg/kg, IP) exhibited persistent changes in microglia morphology and elevated pro-inflammatory mRNA markers in the hippocampus, cortex, and midbrain 7 days after LPS injection, but the peripheral immune response had resolved, as evidenced by the reduction of traditional serum pro-inflammatory cytokines (ex. TNFa and IL-1β) in circulation. Ex vivo serum by the reduction of traditional serum pro-inflammatory cytokines (ex. TNFa and IL-1β) in circulation. Ex vivo serum by the reduction of traditional serum pro-inflammatory cytokines (ex. TNFa and IL-1β) in circulation. Ex vivo serum by the reduction of traditional serum pro-inflammatory cytokines (ex. TNFa and IL-1β) in circulation. Ex vivo serum by the reduction of traditional serum pro-inflammatory cytokines (ex. TNFa and IL-1β) in circulation. Ex vivo serum by the reduction of traditional serum pro-inflammatory cytokines (ex. TNFa and IL-1β) in circulation. Ex vivo serum by the reduction of traditional serum pro-inflammatory cytokines (ex. TNFa and IL-1β) in circulation. Ex vivo serum by the reduction of traditional serum pro-inflammatory cytokines (ex. TNFa and IL-1β) in circulation. Ex vivo serum by the reduction of traditional serum pro-inflammatory cytokines (ex. TNFa and IL-1β) in circulation. Ex vivo serum by the reduction of traditional serum pro-inflammatory cytokines (ex. TNFa and IL-1β) in circulation. Ex vivo serum by the reduction of traditional serum pro-inflammatory cytokines (ex. TNFa and IL-1β) in circulation. Ex vivo serum by the reduction of traditional serum pro-inflammatory cytokines (ex. TNFa and IL-1β) in circulation. Ex vivo serum by the reduction of traditional serum pro-inflammatory cytokines (ex. TNFa and IL-1β) in circulation. Ex vivo serum by the reduction of traditional serum pro-inflammatory cytokines (ex. TNFa and IL-1β) in circulation.
in GWI regulates the persistent microglial response, provide much needed insight into GWI biomarkers and therapeutic targets, and highlight a serum bioactivity assay with the potential to elucidate the pathology and target future treatment in GWI.

2472 Integrated CNS and CV Safety Models in Freely Moving Rodents
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Drug-induced seizure/convulsions represent one of the major problems in safety pharmacology. Although in rat, liability for drug induced tremors/convulsions are explored in traditional observational studies, they are not suited for detection of seizures that do not develop convulsions. We intended to identify seizure-related liabilities using innovative in- vivo methods aimed to combine CNS and cardio-hemodynamic endpoints in one experimental in vivo study, keeping the 3Rs in mind. We applied a radio telemetry platform for CNS and cardiovascular continuous physiological measurements (EEG, hemodynamic, temperature and activity) in combination with an automated home cage behavioral assessment provided through machine learning algorithms, and with measurements of selected biomarkers. For the model validation, male Sprague Dawley rats were dosed with three reference compounds (CERC-301, an A2A/A3 antagonist and pentylenetetrazol) and monitored for 24 hours. None of the animals treated with CERC-301 (0.6, 10 and 20 mg/kg oral gavage) or A2A/A3 antagonist (10, 40, and 300 mg/kg oral gavage) showed seizure activity at any of the selected doses. CERC-301 was well tolerated at the high dose (20 mg/kg) but showed a significant increase in blood pressure at 10 and 20 mg/kg and increased locomotion activity. All the selected doses of an A2A/A3 antagonist affected behavioral parameters (i.e. increased locomotion), and autonomic function (increase in temperature). In both compounds, the spectral analysis revealed a decrease of the power in the delta, alpha and theta bands and an increase in the gamma band. EEG spectral analysis revealed important changes in a large range of frequencies (i.e. 0.5–60 Hz) when compared to values prior to PTZ infusion. The results confirm previously reported observational studies, showing many advantages when using automated home cage behavioral assessment in combination with telemetry recordings and continuous observations for Safety Pharmacology assessments.

2473 Gulf War-Related Organophosphate Exposure Results in Changes in Memory and Neural Plasticity
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During the 1990-91 Gulf War, deployed soldiers had the potential to be exposed to several neurotoxic agents. These exposures are the suspected cause of Gulf War Illness (GWI), a multi-symptom, neuroimmune-based disorder that presents with several neurological-based symptoms including cognitive dysfunction and memory deficits. Previously, using a mouse model of GWI, we found that combined exposure to the stress hormone corticosterone (CORT; 200 mg/L) and the sarin surrogate diisopropyl fluorophosphate (DFP; 4 mg/kg, i.p.) resulted in significant and persistent neuroinflammatory changes throughout the brain. As such, we aimed to investigate whether these changes are associated with changes in cognitive function and neural connectivity that could underlie the cognitive symptoms experienced by veterans with GWI. Here, we used our acute exposure model that is more representative of the veterans’ time in theater, and a more extended model representative of the current condition of veterans with GWI who are 30 years removed from their tours of duty. 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, i.p.) in rats, mimics the long-term illness and ‘flare up’ of symptoms as is reported in GWI. Using these exposure paradigms, we found that Novel Object Recognition was impacted by CORT+DFP acutely, while Novel Object Location was affected in our 5 week model. Interestingly, these changes were accompanied by increases in Ki-67+ cells in the hippocampus following the acute exposure paradigm and a decrease in the expression of genes related to synaptic plasticity (particularly increased NR2A and NR2B glutamate receptor mRNA) both acutely and in the 5 week model. The behavioral changes observed in our model are suggestive of the cognitive and memory impairments experienced in GWI and highlight the potential for these exposures to produce neurobehavioral consequences along with neuroimmune disruption. Furthermore, as both the behavioral and plasticity-related changes differed between our acute and longer-term models, our data suggests the potential for the underlying pathology of GWI to evolve over time.

2474 Assessing the Differential Sensitivities of a Microelectrode Array Assay and a Neurite Outgrowth Assay for Detecting CNS Liabilities Using a Set of Neurotoxic and Seizuregenic Compounds
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Domonic acid is a neurotoxin first associated with the poisoning of 107 individuals and three subsequent deaths on Prince Edward Island in 1987 after these individuals consumed mussels containing this toxin. Due to its neurologic adverse effects, including memory loss, the illness was called amnesic shellfish poisoning. The toxin has since been attributed to the diatom, Nitzschia pungens f. multiseries, which was ingested by the mussels during normal filter feeding. In our lab, we use domonic acid as a tool compound when testing neuromuscular toxicity on a microelectrode array (MEA) platform (Axion Biosystem’s Maestro). Domonic acid completely eliminates all spontaneous spike activity when treated at concentrations >1 µM. When tested in a neurite outgrowth assay, a high content imager (ThermoFisher Scientific CellInsight CX7), domonic acid does not affect cell health or neurite outgrowth when tested up to 25 µM for 72 hours. We tested various neurotoxins and seizurogenic compounds on the MEA platform and in the neurite outgrowth assay to compare results. The assay results strongly indicate that domonic acid did not demonstrate seizurogenic liabilities >10 µM on the MEA but had an IC50 of <0.05 µM for neurite outgrowth when tested in an HCS assay. GABA_a antagonist, picrotoxin, had a seizurogenic MEA profile as low as 1 µM but did not affect cell health and neurite outgrowth at concentrations up to 50 µM. With the results from domonic acid, paclitaxel, picrotoxin and 10 additional control compounds, we have determined that the MEA platform is more sensitive for detecting electrophysiological CNS liabilities than the neurite outgrowth assay. Alternatively, compounds which affect neurons by non-electrophysiological cytotoxic effects can be detected more sensitively with the HCS neurite outgrowth assay than the acute MEA assay. Therefore, an effective overall strategy would be to test compounds for safety in both assays.

2475 Botulinum Neurotoxin Sensitivity of Motor Neurons Differentiated In Vitro
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Botulinum neurotoxins (BoNTs), the most potent toxins known to occur naturally, can enzymatically cleave proteins essential for neurotransmitter release in neurons, especially motor neurons (MNs). The BoNT serotypes A1 and B1 are produced industrially and utilized for the treatment of motor or glabular hyperactivity, as well as in aesthetic medicine. The potency of every produced batch needs to be assessed, resulting in 600,000 mice being used annually for the mouse bioassay. Although the animal experiment is still the gold standard, it often shows high variation and is not representative for human BoNT sensitivity. Available alternatives are often proprietary and their applicability is limited to specific serotypes and preparations. In order to develop a representative model, human induced pluripotent stem cells were used for the generation of MNs that allow screening of MNs with different yields of MNs were generated and analyzed in vitro. Depending on the protocol used, neuronal populations with different yields of MNs were generated and analyzed for their suitability for BoNT potency assessment. Using immunocytochemistry and RT-qPCR, all receptors and substrates for multiple BoNT serotypes were detected at the protein and gene expression level, indicating that the generated MNs could be suitable for the potency assessment of several BoNT serotypes. Sensitivity to BoNT/A1 and B1 of human MNs was compared to the human neuroblastoma cell line SiMa, which is commonly used for in vitro assays, by quantifying the cleavage of the respective substrate proteins. The generated MNs could not only quantify much lower concentrations of BoNT/A1 than SiMa cells, they could also be used to assess BoNT/B1 potency, for which SiMa cells have proven to be unsuitable. In conclusion, the use of MNs might vastly improve current alternative methods for the potency estimation of BoNTs. MoNLightBoNT-Assay development was funded by a grant from the German Federal Ministry of Education and Research (FKZ 0311032A/B).
Microglia are the resident immune cells in the brain. While classically defined by innate immune functionality, microglia also have critical functions during development, including regulating neuronal number and synaptic connectivity. In addition to immune messengers, microglia also respond to gonadal hormones and exogenous molecules. As such, they may be affected by exposure to polychlorinated biphenyls (PCBs), a persistent organic pollutant in the environment and in animal tissue. PCBs have been linked to reproductive and immune dysfunction in humans and have pro-inflammatory and anti-estrogenic effects and was thus chosen for this study. Our hypothesis is that direct exposure of microglia to PCBs will alter their expression of cytokines, at basal states or in response to stimulation by lipopolysaccharide (LPS), in a sex-specific manner. Primary microglia cultures were obtained separately from neonatal male and female rats. Mixed glial cultures were grown and microglia were isolated by mild trypsinization and subcultured. Microglia were then treated for 8 hours with a 0.1 μM, 1 μM, or 10 μM PCB solution or vehicle; 4 hours into PCB treatment, LPS or vehicle was applied to the cultures; RNA was then isolated from the cells and qPCR was performed to analyze Tnf and Il1β expression. While PCB exposure alone did not alter expression of either cytokines, microglial response to LPS was greatly exacerbated by pre-exposure to the 0.1 μM and 1 μM doses, in female and male cells, respectively. This is in agreement with our previously published data on potentiated cytokine response to PCBs in neonates in vivo. Future experiments include analysis of ROS production and protein confirmation. Together, these data demonstrate that male and female microglia are directly affected by xenobiotics and highlight the involvement of microglia in effects of PCBs on in the developing brain by PCBs.

The detection of changes in functional neuronal activity is critical for assessments in neurotoxicology and safety pharmacology. Emerging methods utilizing in vitro cell-based assays of functional neurophysiology have shown promise in identifying neuro-active agents and may demonstrate value for proconvulsant risk assessment earlier in drug discovery. However, mechanistic information can be difficult to obtain as changes in functional neuronal activity may arise due to effects on ion channels, synaptic propagation, intra-cellular signaling, and cell structure. Here, we describe the development and characterization of a simple in vitro assay of synaptic propagation between two distinct neural circuits. First, an easy-to-use, two-compartment silicone insert was added to each well of a 6-well microelectrode array (MEA) plate. Cryopreserved rodent primary cortical neurons were prepared and seeded into each compartment of the silicone inserts. An adeno-associated virus was added to transduce the cells of each compartment with a distinct optogenetic construct (Chronos vs. Chrimson). After two days in vitro, the silicone insert was removed and the networks allowed to mature. The development of functional network activity (e.g., activity, synchrony, oscillations) was monitored every 2-3 days throughout the culture cell period. Activity and synchrony developed first (~7-10 days) within the networks originally defined by the two-compartment silicone insert, followed by the propagation of network activity from one compartment to the other (~14-17 days). Optogenetic stimulation was used to selectively stimulate one network at a time within each well of the plate while the electrophysiological activity was monitored from the second network. The evoked synaptic propagation was evaluated for each well at baseline, after treatment with either the vehicle control or a cocktail of synaptic blockers (CNQX, APV, Bicuculline), and then following washout. The effect of the synaptic blockade was quantified as the probability and delay of synaptic propagation in response to the optogenetic stimulus. These results support the continued development and use of in vitro neuronal models and MEA technology for drug toxicity and safety assessment, evaluation of phenotypic disease-in-a-dish models, and cell development.

Diethylene glycol (DEG) is an industrial solvent with a history of use in or contamination of pharmaceutical products resulting in mass poisonings from the 1930s to the present. DEG poisoning results in a 3-phase toxidrome of metabolic acidosis, nephrotoxicity and hepatotoxicity, and late-stage neurotoxicity. While the acidosis, nephrotoxicity, and hepatotoxicity are well characterized, the compound responsible for neurotoxicity has not yet been identified. SH-SY5Y neuroblastoma cells were seeded into 24- or 6-well plates and treated for 24, 48, or 120 h with DEG or its metabolites 2-hydroxyethoxyacetic acid (2-HEAA) or diglycolic acid (DGA), alone or in combination. Cell death was characterized using annexin V-FITC and propidium iodide flow cytometric analysis, trypan blue dye exclusion, lactate dehydrogenase (LDH) release, caspase-3 activation, and oligonucleosome formation. DEG treatment for 120 h showed nonspecific cell death at 50 and 100 mmol/L. Further evaluation for LDH release and oligonucleosome formation at these concentrations revealed no significant cell death. ≥50 mmol/L DGA induced significant necrotic and apoptotic cell death at 24 and 48 h as evidenced by substantial LDH release, trypan blue exclusion dye uptake, caspase-3 activation, and internucleosomal DNA fragmentation. As DGA exposure time increased (120 h), the DGA concentration required to elicit significant increases in oligonucleosome formation decreased (20 mmol/L). Additionally, longer exposure time (120 h) resulted in significant decreases in the calculated DGA LC50 (LDH release 3.88±2.03 mmol/L; nucleosomal DNA fragmentation 9.30±1.50 mmol/L). These data indicate a time-dependent increase in DGA potency. Additionally, combination treatment for 120 h revealed significant oligonucleosome formation only with DGA alone or DGA combined with either 2-HEAA or DEG. In summary, these results indicate that DGA induces time- and threshold concentration-dependent cell death in SH-SY5Y neuroblastoma cells in vitro.

Disruption of dopamine (DA) homeostasis is a proposed mechanistic link between environmental insults and disease. Environmental insults include dieldrin (DI), which is an organochlorine (OC) pesticide. Another class of OCS are polychlorinated biphenyls (PCBs). Both PCBs and DI persist in the environment posing a public health concern. Specifically, DI has been association with an increased risk of Parkinson’s disease, while PCBs have been linked to neurodevelopmental conditions. These neurological conditions - both neurodevelopmental and neurodegenerative - are characterized by an imbalance of DA, including metabolism and trafficking. DA is metabolized to 3,4-dihydroxyphenylacetaldehyde (DOPAL) by monoamine oxidase (MAO), producing reactive oxygen species. DOPAL is converted to 3,4-dihydroxyphenylacetic acid (DOPAC) by aldehyde dehydrogenase (ALDH). Levels of DOPAC and in vivo data implicate DOPAL, a biogenic aldehyde, as a toxic factor relevant to disease. This includes increased DOPAL:DOPAC ratios in PD patients revealing an imbalance in DA metabolism. Here, the purpose of this study was to characterize the mechanistic link between OCS and DA disruption. The toxicity of OCS was determined using a dopaminergic cell culture model. Additionally, both cellular and mitochondrial ROS was analyzed using MitoSox and CellLight staining. To study DA metabolism, N27 cells were stably transfected with the human dopamine transporter (hDAT), which allowed for rapid uptake of DA and turnover. PCB-52, a major component of PCBs in indoor and outdoor air was studied along with its metabolites 4-OH PCB-52. The metabolite was more toxic and increased mitochondrial and ROS more than the parent PCB compound. DI was also found to increase mitochondrial ROS at non-toxic concentrations in dopaminergic cells. Overexpression of hDAT in N27 cells was found to be an efficient model for studying DA metabolism and to assess changes to DA homeostasis in the presence of OCS. Future work will characterize potential changes in DA metabolism, identify protein targets of DOPAL and/or OC agents, and explore the role of glia in maintaining DA homeostasis.
IC50), as compared to TBBPA (NSC 20 μM, HT-22 50 μM) and BDE-47 (NSC 9 μM, HT-22 50 μM) induced significant increases in apoptosis, although 100 μM TBBPA only induced apoptosis and necrosis after 48 hr. Interestingly, these chemicals also induced cell cycle alterations with increasing exposure time. Upon 24 hr exposure, HBCD (50 or 100 μM) induced significant S-phase arrest which was maintained upon 48 hr exposure. However, only upon 48 hr exposure did BDE-47 (50 and 100 μM) and TBBPA (100 μM) induce significant S-phase arrest. These data demonstrate that BFRs can induce chemical-dependent toxicity in neural cells in vitro, possibly by multiple mechanisms. Further study is needed to determine if BFR-induced neurocytotoxicity would adversely affect learning and memory in vivo.

Neurotoxicity has been linked to exposure to a number of drugs and chemicals, yet efficient, predictive, and minimally-invasive methods to detect it are lacking. Fluid-based biomarkers such as those found in serum, plasma, urine, and cerebrospinal fluid (CSF) have great potential due to the relative ease of sampling, but at present, data on their expression and translation are lacking or inconsistent. Here, we present data on biomolecules that have promise for detection and characterization of neurotoxicity induced by the known neurotoxic agent, trimethyltin (TMT). A single dose of TMT (7 mg/kg, ip) to the rat led to significant alterations in markers of neuroinflammation detectable in CSF, and a proteomic analysis reflected significant alterations in signaling molecules related to neurotoxicity with TMT treatment. TMT samples contained between 29-237 proteins that were significantly different from controls. Network analysis determined that TMT treatment resulted in higher levels of proteins associated with neurological disease and cellular assembly and lower levels of proteins associated with cell survival as compared to controls. These findings provide an opportunity to explore the correlation of these fluid biomarkers with traditional neuropathology and magnetic resonance imaging (MRI) that serve to define TMT-induced neurotoxicity. Our data demonstrate a comprehensive correlation of TMT-induced neuropathology with potential neurotoxicity biomarkers and MRI-based endpoints, findings suggestive of an involvement of specific pathways that can be assessed using peripheral fluids. Supported by NCTR Protocol E0758001. Disclaimer: This presentation does not represent US EPA or FDA policy.

The degree of response to diseases, stressors, but also to toxic chemicals is dependent on the susceptibility of the individual; however, the underlying reasons for these differences remain largely unclear. Inter-individual differences in responses to chemicals can have widespread consequences, as they could tip the scales between efficient drug therapy and life-threatening toxicities. Similarly, in environmental toxicology such differences decide between succumbing or adapting to a chemical threat, potentially extinguishing populations or giving rise to tolerant ones. In particular, for neuroactive chemicals individual effects are difficult to predict, due to the complex etiology of neurological and mental disorders and the multitude of unknown molecular targets. By investigating inter-individual differences in zebrafish larvae, we aim to identify molecular targets of neuroactive chemicals, whilst exploring the reasons underlying variability in responses to these chemicals. We use behavioral measures to sort chemically exposed individual zebrafish larvae based on their sensitivity. Larval locomotor behavior is widely used as a read-out for the assessment of external challenges to the nervous system; however, it is highly variable and difficult to predict at the individual level. Yet, in an initial analysis of unexposed larvae, we found that locomotor activity of an individual becomes consistent from 6 to 7 days post fertilization, with variability lowest when fish encounter sudden darkness. Using this information, we carried out exposures to neuroactive chemicals and sorted the larvae into tolerant and susceptible populations based on their response. Individuals from the different sensitivity categories are currently being subjected to transcriptome analysis to explore the molecular mechanisms that underpin these sensitivity differences. In addition, differentially regulated genes might point to potential molecular targets of the chemicals. Overall, these data will provide mechanistic understanding of toxicological responses that can benefit drug discovery into processes and human health, whilst additionally supporting environmental risk assessment, as high quantities of neuroactive chemicals and other man-made chemicals are frequently detected in the aquatic environment.

Electronic cigarettes (e-cigarettes) are battery-powered devices, that generate an aerosolized vapor from a liquid and are a popular alternative to tobacco products, particularly among the youth. While the neurotoxic effects of cigarette smoke are well-characterized, little is known about the effects of e-cigarette constituents on neuronal damage and neurodegeneration. Thus, we investigated the potential neurodegenerative effects of the e-cigarette constituents, propylene glycol (PG), vegetable glycerin (VG), and PG/VG in the Caenorhabditis elegans model. C. elegans were used to assess the neurodegenerative effects of the e-cigarette constituents, as they have conserved neurons, are green fluorescent protein (GFP) enabled, and are a successful alternative model to study neuronal morphology and degeneration. For this study, C. elegans at the first larval stage (L1) were exposed to dilutions (0-10%) of either PG, VG or PG/VG in nematode growth medium (NGM) agar for 48 hr. Worms were visually observed at 0, 24 or 48 hr. to assess any changes in development and movement compared to normal worms. We observed slower development and movement rates in worms exposed to 5% and 10% of PG, VG and PG/VG with more dramatic effects with PG alone. Neuronal degeneration was evaluated after 48 hr, using a fluorescence microscope, to visualize GFP tagged dopaminergic (DAergic) neurons for morphological changes or loss. DAergic neurons are linked to movement and cognition behaviors. Each worm was scored for absence (0) or presence of morphological changes representing degeneration including: thinning of neuron projections (1); 2-3 bleb formations (2); >3 bleb formations or shrunken soma (3); and loss or breaks in GFP (4). Notable morphological changes were observed in the neurons upon exposure to PG, VG and PG/VG at 5% and 10%; PG exposure alone had the most severe effects, as worms exhibited the greatest amount of neurodegeneration, with average degenerative percentage at 72.86% compared to controls based on the numbering criteria. Overall, findings indicate that individual and combined constituents of e-cigarettes adversely affect the DAergic pathways of C. elegans, initiating neurodegeneration in this species. This data suggests that like traditional cigarettes, constituents from e-cigarettes affect neuronal pathways and cause neurodegeneration. Supported by NYU Dept. funds and NICHD R01ES01563.
Compound safety and efficacy are necessary and complementary components of the drug discovery pipeline, and tools for accurate and rapid screening of both endpoints are vital. iPSC-derived neurospheroids offer a promising in vitro cellular model for early-stage, human-first studies to identify the safest and most effective compounds for pipeline progression and decreased late-stage attrition. Here we developed healthy and patient iPSC-derived neurospheroids in a high-throughput screen to identify compounds that rescue the disease phenotype without inducing neurotoxicity. Neurospheroids in 384-well plates underwent chronic treatment with a library of 296 well-annotated compounds followed by simultaneous multiplexed functional, structural, and viability evaluation for phenotypic rescue and/or neurotoxicity. Functional assessment employed FLIPR-based readouts of neural activity where over 70 individual endpoints were quantified and used to rank-order rescue. Structural and viability assessments were taken with microscopic and ATP-based evaluations that, along with the FLIPR readouts, were used to ascertain the level and concentration dependence of any toxicity. Example results include 1) compounds that showed toxic responses without any phenotypic rescue; 2) compounds that showed various levels of rescue with evidence of toxicity at higher doses; and 3) 27 compounds that showed good to excellent rescue without any functional, structural, or viability-based toxicity. Compounds eliciting phenotypic recovery spanned several cellular pathways including (but not limited to) the serotonergic, dopaminergic, and GABAergic pathways. Further dissection of cholinergic-based rescue revealed that the primary effect was due to inhibiting the action of acetylcholinesterase. Our results demonstrate a highly specific platform for simultaneously uncovering potential neurotoxicity while identifying the most effective compounds capable of restoring neural function from both healthy and patient-derived material. This “human-first” platform brings relevant biology for both safety and efficacy endpoints into early drug discovery, enables toxicity testing on patient derived material, and holds the potential to reduce costs and accelerate development by ensuring that only the most effective and safest compounds are progressed along the development pipeline.

### 2485 Human iPSC-Derived Neural Spheroids Provide a High-Throughput Platform for Early Neurotoxicity Detection

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The current gold standard for pre-clinical CNS testing is the rodent functional observational battery (FOB) which offers safety assessments and dose-setting information for clinical trials. However, serious adverse events uncovered at this point cause a significant loss of time and money. Furthermore, as the FOB depends on visual observations of gross neuromotor abnormalities and early stage in vitro testing often relies on non-functional, viability-based endpoints, neurotoxicity is still one of the primary reasons for toxicity-based withdrawal of marketed compounds. Therefore, in order to progress the most promising and safest compounds to the clinic, we developed and present a high-throughput human-based neural platform coupled with high-speed data analysis to identify CNS liability early in the drug discovery process. Human iPSC neural spheroids showing spontaneous, synchronous activity in 384-well plates were exposed to negative control, seizurogenic, peripheral neuropathy-inducing, and withdrawn compounds. The functional impact of acute (1-6 hrs) and chronic (7-day) compound exposure was assessed via kinetic Ca²⁺ imaging and quantified by extracting over 70 waveform parameters. Holistic analysis showed minimal impact of the negative controls and significant perturbation with 5/5, 12/16, and 13/16 of the peripheral neuropathy, seizurogenic, and withdrawn compounds, respectively. Notably, the peripheral neuropathy compounds in general required chronic exposure to show significant effects and the fingerprint of activity pattern was similar across this class of compounds including increased waveform heterogeneity and variability in peak height and peak rise time. Several interesting observations were also apparent from the withdrawn compound group including 1) The novel finding of pre-clinical toxicity with Minaprine, Carmofur, and Mepazine in a high-throughput screen to identify compounds that rescue the disease phenotype without inducing neurotoxicity. Neurospheroids in 384-well plates underwent chronic treatment with a library of 296 well-annotated compounds followed by simultaneous multiplexed functional, structural, and viability evaluation for phenotypic rescue and/or neurotoxicity. Functional assessment employed FLIPR-based readouts of neural activity where over 70 individual endpoints were quantified and used to rank-order rescue. Structural and viability assessments were taken with microscopic and ATP-based evaluations that, along with the FLIPR readouts, were used to ascertain the level and concentration dependence of any toxicity. Example results include 1) compounds that showed toxic responses without any phenotypic rescue; 2) compounds that showed various levels of rescue with evidence of toxicity at higher doses; and 3) 27 compounds that showed good to excellent rescue without any functional, structural, or viability-based toxicity. Compounds eliciting phenotypic recovery spanned several cellular pathways including (but not limited to) the serotonergic, dopaminergic, and GABAergic pathways. Further dissection of cholinergic-based rescue revealed that the primary effect was due to inhibiting the action of acetylcholinesterase. Our results demonstrate a highly specific platform for simultaneously uncovering potential neurotoxicity while identifying the most effective compounds capable of restoring neural function from both healthy and patient-derived material. This “human-first” platform brings relevant biology for both safety and efficacy endpoints into early drug discovery, enables toxicity testing on patient derived material, and holds the potential to reduce costs and accelerate development by ensuring that only the most effective and safest compounds are progressed along the development pipeline.

### 2486 Cell Differentiation State of SH-SY5Y Cells Determines the Level of Aryl Hydrocarbon Receptor-Mediated Parkin Induction


Parkin is an E3 ligase enzyme encoded by the PRKN gene. One of the main Parkin’s roles is the maintenance of neuronal survival by promoting mitochondrial protein ubiquitination, making it an essential player for cellular mitochondrial integrity. Recent evidence indicates that mitochondrial dysfunction due to Parkin loss is a predominant cause of Parkinson’s disease. Therefore, it is essential to understand the molecular mechanisms that control hPRKN gene expression. Previous results revealed that the Aryl Hydrocarbon Receptor (AhR) induces prkn gene expression in the mouse ventral midbrain, suggesting that this transcription factor also modulates human Parkin expression. The present work determined whether hPRKN gene is under AhR regulation in non-differentiated (ND) and differentiated (DF) SH-SY5Y neuroblastoma cell line. The hPRKN gene promoter was analyzed using the bioinformatics tool JASPAR. SH-SY5Y cells were differentiated using Retinoic Acid 10 μM. To activate AhR cells were treated with 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) 10 nM and the PRKN mRNA was evaluated by RT-qPCR. For transcription validation, 970 bp containing the hPRKN promoter was cloned into a pGL4.10 (luc2) reporter vector and transfected into ND SH-SY5Y cells and treated with TCDD. In-silico analysis showed two AhR response elements located at ~42 bp and ~105 bp from the transcription start site, and thus, the effect of AhR activation on PRKN gene promoter transcription was evaluated. After 10 nM-TCDD treatment, a 2-fold increase in luciferase activity was observed. Next, we sought to determine whether TCDD treatment increase Parkin mRNA levels. Dioxin treatment results in a 2-fold increase on Parkin mRNA levels in ND SH-SY5Y. In contrast, a 10-fold increase was observed in DF SH-SY5Y. In conclusion, our data show that AhR transcriptionally upregulated by AhR and that the magnitude of such induction depends on cell differentiation state. This work was supported by CONACYT grant 280897.

### 2487 ADB-FUBINACA, a Synthetic Cannabinoid, Enhances In Vitro Neuronal Differentiation at Biologically Relevant Concentrations


ADB-FUBINACA is a potent synthetic cannabinoid (SC) displaying 140-fold higher cannabinoid receptor 1 (CB1R) affinity compared to the phytocannabinoid tetrahydrocannabinol (THC). This SC has been associated with excitation and excitotoxic neuronal and cardiac death, and its potential use by pregnant women or women of child-bearing age is especially alarming due to the possible onset of psychotic outcomes or neurodevelopmental disorders in their offspring. However, ADB-FUBINACA’s preclinical toxicological evaluation remains unexplored. This work assessed the effects of this SC on in vitro neurodifferentiation of NG108-15 neuroblastoma x glioma cells. NG108-15 cell differentiation was induced by serum-starved (1% FBS) cell culture medium supplemented with forskolin and retinoic acid. ADB-FUBINACA (kindly supplied by TicTac Communications Ltd, UK) was added either once (day 0), or every 24h for 3 days, at non-toxic, in vivo-relevant concentrations (1pM - 1μM). The resulting effects on differentiation ratios and total neurite length were then measured. Global DNA methylation and histone H3 acetylation were also measured in 3 distinct SC exposure settings: (A) single addition at day 0, sample collection at day 3; (B) single addition at day 3, sample collection at day 6; (C) additions at days 0 and 3, sample collection at day 6. ADB-FUBINACA increased differentiation ratios and total neurite length above control, with no significant differences between single and multiple exposures. Such effects were CB1R activation-dependent, as incubation with the selective CB1R antagonist SR141716A prior to SC exposure reset differentiation ratios to control levels. ADB-FUBINACA increased DNA methylation after treatment (A) (1nM), but not at 10μM, and this parameter in treatments (B) (1nM) and (C) (1μM). Also, 1pM ADB-FUBINACA reduced histone H3 acetylation in treatment (C). Our data highlight the CB1R-mediated enhancement of ADB-FUBINACA-induced neurodifferentiation, while suggesting that this effect may be influenced by global changes in DNA methylation and histone H3 acetylation. However, further research is required to understand the mechanisms involved.
Methyleneoxypropyvalerone (MDPV) is a synthetic cathinone or “bath salt” that may pose special risks for adolescent use and dependence. We have recently investigated adolescent vulnerabilities to MDPV because cocaine and other dopamine (DA) transport inhibitors induce greater behavioral and DA effects in adolescents than adult rats. We found that adolescent rats were more sensitive than adults to MDPV increases in motor behavior, stereotypy and reward using conditioned place preference. MDPV effects on the DA reward system were directly evaluated in a novel analysis by comparing effects on spontaneous phasic and tonic DA release using fast-scan cyclic voltammetry (FSCV). The MDPV molecule was discovered to be electroactive with a unique voltammogram at carbon fiber electrodes using scanning parameters for DA. We resolved its concentration concomitantly with DA using a four-substrate, principal components analysis that included pH shift and background current fluctuations. Background subtracted cyclic voltammograms (CVs) were obtained for MDPV in rat cortex minutes after systemic administration of 1.0 mg/kg sc. These in vivo CVs matched those obtained from MDPV solutions tested in vitro in a flow cell in both the general redox profile and specific voltage ranges of activity. MDPV was detected in nucleus accumbens shell of rats within minutes of sc administration and peaked around 25-30 minutes in both age groups at 0.5 to 0.7 µM. Increases in phasic DA were synchronized with initial increases in detectable extracellular MDPV. MDPV induced effects on phasic and tonic DA in nucleus accumbens shell were enhanced in adolescents relative to adults although drug levels were comparable. We conclude that the greater motor and reward related behavioral effects in adolescents were due to larger DA increases in adolescents relative to adults. These results demonstrate that the adolescent DA system is predisposed to enhanced activation by equivalent brain drug concentrations of this DAT inhibitor. This novel paradigm for real-time, continuous measurement of brain MDPV concentrations may provide insight into pharmacokinetic/pharmacodynamic interactions during the critical early phase of reward system activation.

### 2489 Dugesia japonica Is the Best Suited of Three Planarian Species for High-Throughput Toxicology Screening

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High-throughput screening (HTS) using new approach methods is revolutionizing toxicology. Asexual freshwater planarians are a promising invertebrate model for neurotoxicity HTS because their diverse behaviors can be used as quantitative readouts of neuronal function. Currently, three planarian species are commonly used in toxicology research: Dugesia japonica, Schmidtea mediterranea, and Girardia tigrina. However, only D. japonica has been demonstrated to be suitable for HTS. Here, the two other species were assessed for HTS suitability by direct comparison with D. japonica. Through quantitative assessments of morphology and multiple behaviors, the effects of four common solvents (DMSO, ethanol, methanol, ethyl acetate) and a negative control were scored. Differences relative to adults although drug levels were comparable. We conclude that the greater motor and reward related behavioral effects in adolescents were due to larger DA increases in adolescents relative to adults. These results demonstrate that the adolescent DA system is predisposed to enhanced activation by equivalent brain drug concentrations of this DAT inhibitor. This novel paradigm for real-time, continuous measurement of brain MDPV concentrations may provide insight into pharmacokinetic/pharmacodynamic interactions during the critical early phase of reward system activation.

### 2490 Cadmium Exposure and LincRNA Tuna: A Novel Role in the Placenta

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Cadmium (Cd) is a toxic heavy metal in the environment and a top ten toxicant. Elevated Cadmium exposure can cause developmental restriction, malformation, and spontaneous abortion. While Cd has been shown to alter methylation and gene expression, the mechanisms by which its actions impact birth outcomes are poorly understood. Cd is inefficiently transported to the fetus, and it accumulates in the placenta, suggesting that these negative outcomes are a consequence of disrupted placental function. To understand how Cd impacts placental function and modulates fetal growth, we performed RNA-seq on control and Cd exposed mouse placentae exhibiting fetal growth restriction. RNA-seq revealed upregulation of Tc11 Upstream-Associated LinCRNA (Tuna) as the top differentially expressed transcript. Tuna is a poorly characterized IncRNA. Tuna has been shown to be critical for neuronal differentiation and a crucial contributor to both oncogenic and tumor suppressive mechanisms. But, within the placenta, there has been no indication that Tuna is normally expressed or functional at any developmental stage. Our findings from RNA-seq and validated through qRT-PCR confirm that Tuna is poorly expressed in control placentae, but is activated in response to Cd exposure, a result that is reproduced in cultured trophoblast cells upon Cd treatment. While the physiological outcomes of Tuna activation within the placenta are not yet known, IncRNAs have been shown to be master regulators of gene expression, actively contributing to the control of epigenetic states as well as establishing RNA-protein complexes. Our primary hypothesis is that the activation of Tuna expression by Cd within the developing placenta leads to the formation of protein complexes capable of disrupting normal patterns of gene expression, contributing to impaired placental function and fetal growth restriction. To study the function of Tuna, we will use over-expression and knock-down approaches in cultured cells. We are currently performing experiments to validate qRT-PCR, pyrosequencing, and RNA-seq to analyze gene transcription and epigenetic states of Cd exposed, control, and Tuna overexpressing cells. This will allow us to determine the function of Tuna within the placenta and further understand the role Cd-activated Tuna expression may have in the negative birth outcomes associated with Cd exposure.

### 2491 Sex-Specific Extracellular Matrix Remodeling during Adipogenic Differentiation by Gestational Bisphenol A

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Bisphenol A (BPA) is an endocrine disrupting chemical (EDC). We have demonstrated that gestational BPA exposure increases the adipogenic ability of primary fetal female, but not male preadipocytes. However, whether early differentiation signals define this enhanced differentiation ability remains unknown. We hypothesized that BPA induces a transcriptomic profile during early differentiation that results in higher adipogenic potential and that this profile is sex-specific. Primary ovine fetal preadipocytes obtained from control (C) and BPA-exposed pregnancies (n=6/group) were used for in vitro differentiation using dexamethasone and rosiglitazone. Cells were fixed and lipids analyzed using Illumina TruSeq (false discovery rate <0.05 and fold change >2), individual samples (n=24) were compared as follows: 2 groups (C vs. BPA); 2 sexes (female (F) vs. male (M)); and 2 time points (0h vs. 48h). At 0h, less than 15 genes were differentially expressed between the C and the BPA-exposed groups within sex, but in BPA-F, extracellular matrix (ECM) remodeling genes were overexpressed and collagen So3 were upregulated. At 48h, BPA-F preadipocytes had higher lipid accumulation compared to C-F and BPA-M groups; BPA-F preadipocytes had 45 up- and 109 down-regulated genes vs. C-F and BPA-M preadipocytes had 10 up- and 477 down-regulated genes vs. C-M. Pathway analysis revealed that triglyceride and glycerocephospholipid metabolism were the most upregulated pathways in BPA-F. Downregulated pathways were associated with ECM organization in both BPA-F and BPA-M. Sex-specific comparisons had more differentially expressed genes within the BPA than the C group (555 vs. 100, respectively). At 48h, in BPA-F, genes were upregulated in association with lipid accumulation and down-regulated in association with ECM remodeling, such as collagen (404, 422 fold increases) and metalloproteinases (MMP13 and ADAMTS9) when compared with C-F. Transcriptomic alterations in BPA-F preadipocytes at 48h suggest an accelerated adipogenic transition. Notably, BPA also affected adipogenic differentiation by altering genes associated with ECM components which may contribute to accelerated adipogenesis in early differentiation stages. Supported by 1K22ES026208 and R01ES27963 to AV-L.
Bisphenol S (BPS) is a bisphenol A-analog with endocrine disrupting properties. BPS has been shown to alter metabolic function in vitro and cause hyperactive behaviors in zebrafish, but in-depth toxicological research, particularly following developmental exposure, is scarce. The purpose of this study was to examine the reproductive and developmental effects of BPS using the nematode strain C. elegans and non-obese diabetic excluded flora mice (NODEF). It was hypothesized that BPS exposure would result in reproductive toxicities, reduced lifespan and behavioral deficits in both C. elegans and NODEF mice. We first evaluated the effects of developmental BPS exposure on fertility and lifespan in the N2 strain of C. elegans and NODEF mice. We synchronized embryos were treated with 0, 0.1, 1, 5, or 10 µM BPS for 48 hours. Worms treated with either 5 or 10 µM BPS had a significant decrease in their average lifespan, along with an increase in embryonic lethality. Although not statistically significant, the number of eggs laid also decreased in a concentration-related manner. However, BPS-treated worms had a longer or delayed egg-laying period. The control worms stopped laying eggs after day 5, while BPS-treated worms at concentrations of 1, 5 and 10 µM continued to lay eggs at day 6, 7 and 8, respectively. Generational exposure at all concentrations resulted in a decrease in lifespan and viable offspring. To further examine the developmental effects, female NODEF mice were mated and exposed to 3 µg/kg BW BPS (equivalent to 2.5 µM BPS in C. elegans exposure) throughout gestation. Pregnant females treated with BPS exhibited a higher incidence of type 1 diabetes (Blood glucose level > 250 mg/dL); however, they were more likely to get pregnant following 5 days of breeding. For the offspring, the BPS-treated litters had a higher survival rate. To examine postnatal behavioral effects, offspring of the treated mice underwent behavior testing at 3 and 12 weeks of age. Both the novel object and tail-suspension tests showed an increase in hyperactivity and an impairment of memory. Overall, this study demonstrates that BPS can alter metabolic function and regulate developmental tox-
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Testing for developmental toxicity according to the current OECD guidelines requires large numbers of animals, making these tests very resource-intensive and time-consuming as well as raising ethical concerns. Over the past 20 years, several alternative in vitro assays have been developed, but these often suffered from low predictability and a lack of mechanistic information. To improve the current in vitro developmental toxicity screening, we developed ReproTracker, a human induced pluripotent stem cell (hiPSC)-based biomarker assay that follows the differentiation during early embryonic development. Here, hiPSCs were directed to differentiate into the two germ layer specific cell types, hepatocytes and cardiomyocytes. During differentiation, expression of the pluripotency marker OCT4 decreased, while early mesoderm and endoderm developmental markers, BMP4 and FOXA2, were significantly induced. Upon further maturation of heart and liver tissues, expression of the cardiomyocyte-specific marker MYH6 and liver-specific marker AFP were observed in the respective tissues. Alterations in the expression pattern of these biomarker genes upon chemical exposure indicated perturbation of stem cell differentiation and thereby teratogenicity. To illustrate the applicability of the assay, we tested 53 known teratogenic and non-teratogenic compounds at non-cytotoxic conditions during cardiomyocyte and hepatocyte differentiation. ReproTracker identified most of the in vivo teratogenic compounds (e.g. thalidomide and valproic acid), which markedly disrupted morphology, functionality and the expression pattern of the cardiomyocyte genes in both cell types. Non-teratogenic chemicals (e.g. folic acid and thiamine), generally had no effect on the morphology of differentiated cells, nor on the expression of the biomarker genes. The assay demonstrated high predictability (83%), sensitivity (90%) and specificity (77%) as compared to the in vivo classification. In conclusion, here we present a novel in vitro biomarker assay that combines morphological readouts and functional readouts for cardiomyocytes and expression changes of the selected biomarkers in different embryonic lineages to reveal the developmental toxicity of chemicals in a time-dependent manner.

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Acute exposure to ozone (O3) during implantation receptivity impairs fetal growth in both male and female offspring. Altered fetal weight gain is a hypothesized contributing factor in the increasing prevalence of adult cardiometabolic disease by modulating the sensitivity to a poor diet. To investigate this phenomenon in our model of O3-induced growth restriction, pregnant Long-Evans rats were exposed to 0.8 ppm O3 or filtered air during gestational days 5 and 6 (4 h/d). In adolescence, male and female offspring were fed a 3-day high-fat diet (HFD) to assess their metabolic flexibility. In offspring from O3-exposed dams, HFD increased caloric intake and reduced energy expenditure in both sexes. In males, this caloric imbalance resulted in reduced hepatic triglycerides, increased circulating cholesterol, and increased body adiposity. Such extrahepatic lipid mobilization was not prevalent in the female offspring (O3+HFD). Postnatal HFD challenge in female offspring from O3-exposed dams altered the expression of genes related to insulin signaling, gluconeogenesis, lipolysis, and genes involved in both the signaling and regulation of bile acid status in the liver. Ingenuity Pathway Analysis in the hepatic gene set reported Hepatic Cholesterol, FXR/RXR Signaling, and AMPK Signaling as top canonical pathways altered. Unlike in the male offspring, females were unable to activate AMPK phosphorylation, increase Sirtuin 1, or reduce p300 in the liver after three days of HFD, highlighting a potential divergence in the hepatic metabolic response to HFD between the sexes. Together these findings indicate that acute exposure to ozone early in pregnancy programs the postnatal susceptibility to a calorically dense diet high in saturated fat. However, the specific impacts of this appear to be driven by sex differences in hepatic metabolism in the offspring. Does not reflect US EPA policy.
2501 A Neuroanatomical Mechanism Linking Perinatal Chemical Exposure to Prostate Smooth Muscle Hyperactivity and Altered Voiding Function

In the fetal prostate. This is the first evidence that intrauterine chemical tone incites abnormal urodynamics in adult male mice, including increased and 14 weeks of age and collected prostate tissue to stain for noradrenergic axon density, which have been directed towards the influence of early life events on urinary physiology in advanced age. Here, we identify intrauterine environmental contaminant 2,3,7,8 tetrachlorodibenzo- as a modifier of adult voiding function and risk factor for male LUTD. To model environmental chemical exposures, we exposed pregnant mice to the environmental contaminant 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD, 1 µg/kg, i.o.), coinciding with initiation of lower urinary tract development in male fetuses. When aged male pups to embryonic day (E) 17.5, postnatal (P) day 9, and 14 weeks of age and collected prostate tissue to stain for noradrenergic axons via immunohistochemistry. RNAseq was performed on E16.75 fetal prostate to identify dysregulated neurotrophic factors. Prostate muscle sensitivity was measured using genetically encoded calcium receptors and tissue bath. Urinary frequency was measured using cystometry. Fetal TCDD exposure incites abnormal urodynamics in adult male mice, including increased urinary voiding frequency. TCDD also enhances adult prostate sensitivity to electrically evoked muscle contraction, suggesting increased autonomic tone. IUL TCDD exposure permanently increases noradrenergic axon density beginning in the fetal period and persisting into adulthood. These changes are accompanied in the development of a neurotransmitter, Arternin (Artn), in the fetal prostate. This is the first evidence that intrathecal gene expression can reprogram prostate neuroanatomical development and drive prostatic smooth muscle hyperactivity in adulthood, which may create a susceptible phenotype for aging-related male lower urinary tract dysfunction.

2502 Embryonic Exposure to Camel Snus Leads to Skeletal Defects in Early Development
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The U.S. Department of Health and Human Services reports greater than 100,000 babies have died in the last 50 years due to complications from smoking throughout pregnancy. This statistic neglects those babies that survive in utero tobacco exposure, but exhibit physical deformities, specifically isolated to the skeleton. In the US, 10-12% of women still consume tobacco products during pregnancy, however, opt to alternatives to conventional cigarettes. These alternative products bear a “harm-reducing” label and are advertised as having lower tar and nicotine content, including non-combustible products such as Snus. Our prior in vitro work detected hypomethylization in human embryonic stem cells when differentiated toward bone-forming osteoblasts upon smokeless Snus tobacco extract exposure, suggesting that exposure in utero may still carry risk for other ailments, including bone health and development. Currently, the ramifications of in vivo Snus exposure towards embryonic skeletal development exposed to tobacco remain unclear. To assess the effects of maternal exposure to Camel Snus on the developing skeleton, pregnant dams were injected with Snus tobacco extracts at E6.5 and E8.5. Embryos were harvested at E17.5 to assess skeletal morphologies. Embryos were stained with Alizarin Red S/Alican Blue to identify calcified bone and cartilage, respectively. Preliminary work suggested irregularities in the long bones, such as the forearms and femurs. Upon further analysis, lengths of forearms were shown to be shorter and wider in diameter compared to controls but showed no change in calcified bone lengths. However, femurs demonstrated an increase in the length of calcified bone in Snus-exposed embryos, while overall morphology of the long bone was unchanged. Other phenotypes in the forelimb include irregular "cupping and fraying" in exposed embryos. These seemingly minor changes to bone formation can cause detrimental effects later in life, as commonly seen in epidemiological studies that show an increase of osteoporosis and other related bone diseases in patients born to smoking parents.

2503 Effects of Toxicological Test Process on Juvenile Animal Development

In medical field, the need for safety testing of drugs treating children has been continuously demanded. Adults and children may have different tolerances or mechanisms of toxicity due to their differences in drug sensitivity. So importance of guidelines requiring safety and efficacy of drug for children is emerging because of about 70% of drugs currently on the market are marketed based on non-clinical and clinical trial results from mature individuals. In line with these trends, we continued to conduct toxicity tests on juvenile animals and investigated the impact of these toxicological treatments on juvenile animals. The effect of toxicological treatment was confirmed by using 10-12 weeks old rats which one group of rats that received oral administration of injection water for 8 weeks from 3 weeks after birth and other group of rats that received water orally for 4-6 weeks after birth. As a result, we found a significant increase of weight gain in group of rats that were treated for 8 weeks from 3 weeks after birth. It has been confirmed that the overall health effect of toxicological treatment is insignificant except in weight gain by comparing the relative weights of other organs, (brain, heart, kidney, liver, etc.) or organ sizes. The measurements showed some slight changes which were within the normal range. These results showed that our toxicity test process for juvenile animals could confirm stable and consistent results in toxicity test.

2504 Effects of Arsenic Exposure on the Placenta and Amniotic Fluid in a Mouse Model

Prenatal arsenic exposure has been linked to various negative health outcomes including increased risk of infectious disease in the first year of life; however, the effect of prenatal arsenic exposure on the placenta and its role in immune programming is unknown. Exposure to arsenic in excess of the WHO standard (10 µg/L) for drinking water affects more than 140 million people worldwide. Effects of chronic arsenic exposure are well characterized with increased risk of cancer among the most common. Evidence points to an early immune suppression after prenatal arsenic exposure but the extent to which this then impacts the individual and its associated immune cells, is involved remains unclear. Therefore, we chose to investigate the effects of pre-conception and prenatal arsenic exposure on the amniotic fluid and placenta in a mouse model. C57Bl/6 mice were exposed to 0 or 100 µg/L sodium (meta) arsenite in drinking water from two weeks prior to mating until gestation day 18. In utero tissues were collected. Flow cytometric analysis indicates significantly increased number of total viable cells and mesenchymal cells in the placentas of exposed fetuses. Although not significant, we also observed a trend of decreased Sca-1 expression (a marker of stemness) in trophoblast cells of exposed placentas which could indicate that exposed cells will not infiltrate as well as they should. Analysis of cytokines in the amniotic fluid indicates that exposed male pups had significantly less chitinase 3-like 1 and low density lipoprotein receptor (LDLR) than controls whereas exposed female pups had significantly more chitinase 3-like 1 than their control counterparts. As expected, LDLR was significantly higher in males compared to females (control or treated). Previous reports in a mouse model deficient for LDLR indicate that increased chronic inflammation due to decreased cholesterol uptake leads to atherosclerotic plaque formation. Similarly, chitinase 3-like 1 is involved in activation of the immune system. Therefore, too little LDLR and chitinase 3-like 1 could lead to a state of immune suppression. We are further investigating signaling in the placenta using RNA microarray. Importantly, results from the current study could result in the pursuit of early intervention strategies to reduce the long-term effects of early life arsenic and other heavy metal exposures.

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Polycyclic aromatic hydrocarbons (PAH), including benzo[a]pyrene (BaP), are implicated in many adverse outcomes in offspring of exposed parents such as: neural tube defects, growth defects, cardiovascular toxicities, endocrine disruption, childhood cancers and infertility. However, the molecular mechanisms for the developmental and multi/trans-generational effects associated with PAH exposures has yet to be fully elucidated. Previously, we found significant developmental effects of BaP exposure on survival and developmental deformities in AB strain zebrafish larvae. A goal of this study was to characterize the transcriptomic and epigenetic changes associated with preconceptional exposure to BaP. To determine the role of the aryl hydrocarbon receptor (AhR), AhR2-null (ahr2−/−) zebrafish were also used. To accomplish these goals, adult SD and AhR2-null (ahr2−/−) zebrafish were fed 78.3 or 708 µg BaP/g diet (measured) at a rate of 1% body weight twice/day (1.6 or 14 µg BaP/g fish/day) for 21 days. At the end of the 21-day parental exposure, fish were spawned using a crossover design, and the effects in F1 & F2 generations were monitored. BaP exposure had no significant effect on fecundity in the SD strain. In the F0 SD line, there was no significant effect of BaP exposure to adult behavior, but in the F1 adults following high BaP parental exposure, there was significantly reduced female open field behavior (velocity, distance travelled). Larval behavior (96 hpf) assessed through the light/dark test was significantly altered in both the F1 and F2 generations of 5D fish exposed to 78.3 µg BaP/g diet. Furthermore, F1 SD fish were significantly shorter in length at 1 and 3 months of age. Transcriptomic analysis did not detect any significant changes in gene expression patterns in any of the treatments. However, differential methylation was observed in the F1 generation in response to pre-conceptional BaP exposure. The differential methylated regions were localized to imprinted regions. Transcriptome F0 fish did not produce eggs in any treatment or control group. In BaP exposed ahr2−/− F0 fish, the male but not female fish displayed significantly reduced open field behavior. These results demonstrate that dietary BaP exposure causes adverse developmental outcomes in multiple generations. Research is supported by NIH ES12165301.

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One of the main challenges in using non animal approaches for risk assessment is the determining the appropriate cell line(s) for in vitro studies and that are representative of the biology. i.e. express the relevant biological processes underlying the toxicological endpoint of interest. We aimed to address this challenge by combining publicly available transcriptomics data and targets for developmental and reproductive toxicity (the DARTable Genome) to determine a minimal set of cell lines with relevant biological coverage for subsequent in vitro studies. We developed a pipeline to annotate the DARTable Genome (~5k genes) which represents the comprehensive set of molecular initiating event and transcript/proteomic biomarkers for DART and includes proteins expressed during development and knockout or mutations that affect developmental effects. We then developed a pipeline to query and analyse data baseline RNA-seq data of 934 human cancer cell lines from the Cancer Cell Line Encyclopaedia (CCLE). Following normalisation of the CCLE dataset, we used 3 metrics to determine the most suitable cell lines to use for subsequent in vitro studies: cell lines that expressed the highest number of DARTable genes, cell lines with the highest number of targets at maximum per-gene expression and cell lines with the highest number of DARTable genes expressed above per-gene average expression. Using this approach, we identified human rhabdomyosarcoma (RD) and human ovarian carcinoma (OVCA3) cell lines as having enough biological coverage for subsequent in vitro studies, and we demonstrate the utility of using a knowledge driven approach to select relevant in vitro cell lines.

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As brominated flame retardants (FR) were phased out in the mid-2000s, use of alternative chemical FR, including aromatic phosphates (AP), steadily increased. Two APs, triphenyl phosphate (TPHP) and isopropylated phenol phosphate (IPP), are used in combination with other chemical FR as treatment to consumer products including foam-based furniture and infant products. TPHP and IPP are leaching into the environment with detectable levels found globally in indoor dust, indoor/outdoor air, aquatic biota and food. Human exposure is worldwide with the most common sources of exposure being contaminated food, drinking water, and house dust. This is concerning since there is little toxicity data available with which to evaluate potential risk from exposure of TPHP and IPP. To address this knowledge gap, we evaluated the toxicological effects of TPHP and IPP in a pre-and postnatal (perinatal) study in rats. TPHP and IPP were administered via dosed feed (in separate studies) at concentrations of 1000, 3000, 10,000, 15,000, and 30,000 ppm to timed-mated Sprague Dawley (IACUC Sprague Dawley SD®) rats from gestation day (GD) 6 through postnatal day (PND) 56. At the time of weaning, pups continued exposure to the respective dam’s group until termination of study. Assessments of TPHP and IPP effects in this study included puberty, thyroid hormones, cholinesterase activity and brain inflammation using marker translocator protein 23 kDa (TSP2). F0 female survival was lower at ≥15,000 TPHP and ≥10,000 IPP prior to 15 days of age. In litter survival PND 4-28 were observed at ≥10,000 ppm TPHP and ≥3000 ppm for IPP, respectively. Treatment-related effects were observed in both dams and pups for body weight, organ weight and cholinesterase activity. For both, TPHP and IPP a decrease in offspring body weight gain was observed at ≥10,000 ppm ranging from 7.8%2 in 6-20% decrease, respectively. A delay (≥2 days) in balanopreputial (BPS) at 1000, 3000 and 10,000 ppm TPHP and 1.6 day in 3000 ppm IPP was observed in males. In female offspring, vaginal opening (VO) was delayed ~2 days at 3000 ppm TPHP and IPP groups. No animals in the 15,000 ppm or 10,000 ppm (females only) TPHP treatment group achieved BPS or VO during the time of examination. With TPHP exposure, acetylcholinesterase (AChE) activity was significantly decreased at ≥10,000 ppm in dams and decreased activity was observed in offspring ≥10,000 ppm though it did not reach statistical significance. AChE activity in whole blood collected from dams and pups decreased (8-50%) in a dose-dependent manner across all IPP exposures. There was no significant increase in TSP2 expression in dams; and some small increases were noted in pups exposed to 10,000 ppm TPHP and IPP. Additionally, preliminary data indicated that both TPHP and IPP crossed the placental barrier as well as the blood brain barrier as indicated by uptake in the brain. This work suggests the LOAEL for TPHP was 3000 ppm and for IPP was 1000 ppm for which no adverse outcomes were currently underway at the NTP to characterize longer-term effects on reproduction, development and developmental neurotoxicity following exposure to TPHP and IPP.

**2510 Does Nrf2 Play a Role in the Developmental Toxicity of the Sulfate Metabolite of 3,3'-Dichlorobiphenyl (PCB-11)?**

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The environmental pollutant 3,3'-dichlorobiphenyl (PCB-11) is a lower-chlorinated polychlorinated biphenyl (PCB) congener present in air and water samples. Both PCB-11 and its metabolite, PCB-11-Sulfate, are detected in humans, including in pregnant women. Previous research in zebrafish (Danio rerio) has shown that exposures of 1-3 day post-fertilization (dpf) impede Cyplα enzyme activity at 4 dpf and increase hepatic neutral lipid accumulation at 15 dpf. As inhibition of the catalytic cycle of the Cyplα enzyme has been known to generate reactive oxygen species, this research explored whether oxidative stress contributes to PCB-11-Sulfate embryotoxicity via the master-regulator of the adaptive response to oxidative stress, Nrf2a, and its responder, the nuclear factor erythroid-2 (Nrf2), particularly in the redox-vulnerable pancreatic β-cells. Transgenic embryos expressing GFP in pancreatic β-cells (Tg(ins:GFP)) were crossed with wildtype or mutant nrf2a/fh318/fh318; Tg(ins:GFP) embryos with mutations in the DNA binding domain of Nrf2a and bred to homozygosity. Embryos were exposed to 1.44 dpf to 15,20 µM PCB-11-Sulfate or 0.05% DMSO and at 4 dpf morphology and hepatic Cyplα enzyme activity were captured in vivo with the ethoxyresorufin-O-deethylase (EROD) assay. In a separate experiment, wildtype and mutant transgenic embryos were exposed from 1-15 dpf to either 0.2 µM PCB-11-Sulfate or 0.05% DMSO, and at 15 dpf larvae were imaged for overall morphology and the β-cell cluster in the primary islet of Langerhans, and collected for fatty acid analysis. All experiments were repeated 6 times. Consistent with previous results, at 4 dpf wildtype embryos exposed to 20 µM PCB-11-Sulfate displayed normal morphology and a significant 47% decrease in Cyp1a enzyme activity. Nrf2a mutant embryos exposed to 20 µM PCB-11-Sulfate also developed normally, with a significant 42% decrease in Cyp1a enzyme activity. Tg(ins:GFP) no differences in survival or growth were observed between exposure groups. Mutant larvae exposed to 0.2 µM PCB-11-Sulfate experienced a significant 12% increase in β-cell area of the primary islet. No differences were observed in the relative percentage of fatty acids between larvae exposed to DMSO or 0.2 µM PCB-11-Sulfate, but mutant larvae had a significant decrease in monounsaturated fatty acids compared to wildtype fish. These findings suggest Cyp1a enzyme inhibition from PCB-11-Sulfate exposure is unlikely to cause oxidative stress in the liver, but that lack of functional Nrf2a impacts metabolically-sensitive endpoints. This work was supported by R35ES030975 and R01ES025748.

**2511 Placental ABCB5/MRP5 Transporter Regulates Trophoblast Cell Fusion and Hormone Production**


Syncytiotrophoblasts arise from the fusion of cytotrophoblasts, a process regulated by cyclic nucleotide signaling. Syncytiotrophoblasts express the multidrug resistance-associated protein (MRP/ABC) family, a transporter known to regulate the intracellular concentration of cyclic nucleotides through active efflux. We sought to evaluate the physiological role of MRPs in regulating cytotrophoblast fusion and hormone production and 2) the ability of MRPs to efflux the placental toxicant cadmium. Placental explants obtained from healthy, term pregnancies were treated with vehicle (DMSO) or the MRP inhibitor MK-571 (25 µM) for 24 hr. Human BeWo b30 trophoblasts were treated with vehicle (DMSO), MK-571 (25 µM), or various cyclic nucleotide modulators including 8-Bromo-cAMP (100 µM), forskolin (10, 25 µM), and IBMX (200 µM). Markers of trophoblast cell fusion (GCM1; syncytin 2) and hormone secretion (hCGα and hCGβ) were quantified by qPCR. Stable BeWo b30 cell lines with reduced expression of MRPs were generated using targeted shRNAs and treated with vehicle or cadmium chloride (0-10 µM). Changes in cell size and expression of the tight junction protein E-cadherin were quantified using immunofluorescence. Cadmium concentration was measured by ICP/MS and toxicity determined by protein and glandular staining. Decreased accumulation of cadmium by MRP5 expressing trophoblast cells with MK-571 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 (GCM1; hCGα, hCGβ, syncytin 2) by 20-200%. Similarly, MRP inhibition with MK-571 further enhanced syncytialization and hormone markers following 1) stimulation with the permeable cAMP analog 8-Bromo-cAMP (2- to 6-fold), 2) activation of cAMP synthesis with forskolin (20-160%), or 3) inhibition of cAMP/cGMP breakdown by IBMX (50-130%). In response to treatment with cyclic nucleotide modulators, induction of GCM1, syncytin 2, hCGα, and hCGβ mRNA expression was higher in MRPs knockdown (KD) cells compared to control cells. Furthermore, MRPs KD cellswere 35% larger in size due to greater numbers of multinucleated cells and exhibited decreased staining of E-cadherin, pointing to enhanced syncytialization. Notably, knockdown of MRPs did not affect intracellular concentrations of cadmium nor its cytotoxicity. In conclusion, MRP transporters participate in trophoblast cell fusion, which likely represents a novel mechanism regulating placentalization. Supported by R35ES030927, R01ES029275, T32ES007148, P30ES005022, UL1TR000317.

**2512 Profiling of Heavy Metal Transporters and Stress Response Genes in Human Placentas**

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Gestational exposure to heavy metals, including cadmium, lead, and arsenic, has been associated with a number of perinatal complications and developmental disorders. The placenta can be a conduit for fetal exposure to metals as well as a target of metal toxicity. Understanding metal transporters in the placenta, responses to heavy metals are determined collectively by the extent of cellular uptake via solute carriers (SLC) and detoxification by metallothionein (MT) metal-binding proteins. In the current study, we sought to characterize the expression of SLC metal transporters and MT genes in healthy human placentas from mid-term to term pregnancy. Using existing gene-expression datasets (GSE124282), differences in mRNA expression in human primary cytotrophoblasts were evaluated at mid-term (18-22 weeks, N=4) and term (38-40 weeks, N=4).
1, 7, and 14. In order to further phenotype and characterize the populations, we performed single cell and bulk RNAseq of flow sorted cells. We found both macrophages and T cells were present in the exocrine and endocrine portions of the pancreas as early as e21 and increased in number through PD14. A subset of macrophages, H1S58B3/F4/80, and Cd4+ T cells were expanded by IUGR at PD7. Interestingly, though the number of immune cells returns to normal by PD14, immune cell activation persists as demonstrated by RNAseq and IHC. Finally, using an IL4 neutralizing antibody, the increase in islet immune cells was attenuated thus suggesting these populations are important to the development of type 2 diabetes. This research has furthered our understanding of resident pancreatic immune populations and suggest macrophages and T cells participate in the development of type 2 diabetes induced by IUGR.

**2515 Early-Life Methylmercury Exposure Impairs Adult Flight Muscle Morphogenesis and Performance in Drosophila**


Methylmercury (MeHg) is a developmental toxicant. Recent studies using the Drosophila model show that MeHg can perturb developing muscle and impair flight performance as a functional readout of muscle integrity. However, the underlying cellular mechanisms that mediate myotoxicity are not fully understood. We have previously shown that 10 µM MeHg exposure to larval flies can disrupt developing myoendinous junctions (MTJ), producing myospheres in the developing indirect flight muscles (IFMs) at the pupal stage, by a mechanism that is partially mediated by the NG2/CSPG4 homologue, kon-tiki (kon) - a muscle-specific factor required to establish the MTJ. While exposure to 0 - 5 µM MeHg is insufficient to induce MTJ phenotypes in IFMs, latent impairments in adult flight are observed. Here, we further examined adult muscle phenotypes resulting from early life exposure to MeHg, focusing on the MTJ. Wildtype Canton S. larvae were fed 0, 2.5, or 5 µM MeHg food until eclosion. The effect of MeHg on MTJ-related gene expression during pupal development was assessed via RT-PCR analysis. Upon eclosion, adult flies were washed by sex and transferred to standard food bottles for 11, 30, or 40 days. Flight ability was then assayed by measuring landing height of flies dropped into an adhered-line vertical column. Adult muscle morphology was assessed using fluorescence microscopy. The average landing height from the top of the column (Lz SEM) at 11-day PE was altered by 5 µM MeHg treatment for males and females, which coincided with aberrant MTJ attachment phenotypes in adult muscles. Flight impairment persisted to 30- and 40-day PE timepoints, but age x treatment effects did not reach significance. Expression of kon was reduced by more than 30% relative to untreated controls at a critical window of MTJ formation earlier in life, during pupal development. Altogether, the current data set suggests that developmental exposure to 5 µM MeHg perturbs the adult MTJ and reduces flight ability, yet does not exacerbate the natural decline in flight performance with age. Ongoing studies will investigate if MTJ morphology at 30 days PE is correlated with observed impairments in flight and will further interrogate the contribution of kon to mediate this effect. In the context of MeHg toxicity, this work underscores that developmental origins of adult onset disease can be investigated using the Drosophila model and points to the developing MTJ of Drosophila as a sensitive target of MeHg.

**2516 Mono-(2-ethylhexyl) Phthalate-Induced Testicular Injury Recruits Peritubular Macrophages That Aid in Recovery of Spermatogenesis**

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Peripubertal exposure of male rodents to the phthalate metabolite mono-(2-ethylhexyl) phthalate (MEHP), a widely used plasticizer, causes testicular inflammation, spermatocyte apoptosis, and disruption of the blood-testis barrier. MEHP-induced inflammatory response in the testis includes an infiltration of macrophages and neutrophils, although the cause and purpose of this response is unknown. Recently, a population of testicular macrophages phenotypically distinct from those resident in the testis interstitium was described in mice. Testicular peritubular macrophages aggregate near the spermatogen- al stem cell niche and are believed to stimulate their differentiation. We hypothesize that if testicular peritubular macrophages do indeed stimulate spermatogonial differentiation, MEHP exposure would result in an increase of peritubular macrophages to stimulate the replacement of lost spermatocytes. Male rats were exposed to 700 mg/kg MEHP or corn oil via oral gavage at postnatal day 28 and euthanized at 48 hours, 1 week, or 2 weeks later. Whole adult testes were prepared for fluorescent confocal microscopy. Sections were visualized using a Zeiss scanning confocal microscope. In response to MEHP...
exposure, the presence of peritubular macrophages increased by six-fold after 48-hours and remained elevated by two-fold two weeks after exposure. A corresponding increase of differentiating spermatogonia was observed two weeks after MEHP exposure. Pretreatment with chloronate liposomes was found to inhibit the MEHP-induced increase in the numbers of peritubular macrophages and resulted in a consequent three-fold decrease in number of PLZF-positive peritubular macrophages. These observations have recently gained attention in the field of testis biology because they are thought to play a critical role in the maintenance of the spermatogonial niche under normal physiological conditions in mice. Our findings are significant in that it is one of the first demonstrations that peritubular macrophages are specifically recruited by peritubular injury, as reflected by the large-scale loss of spermatocytes. Furthermore, inhibition of increases in the numbers of peritubular macrophages resulted in decrease in number of PLZF positive undifferentiated spermatogonia. The insights gained from these novel findings indicate that peritubular macrophages play a significant functional role in facilitating the efficient repair and recovery of spermatogenesis after MEHP-induced testicular injury.

2517 Clomifene and Assisted Reproductive Technology in Humans Are Associated with Offspring Epigenetic Alterations in Imprinted Control Regions
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Singleton births from assisted reproductive technology (ART) have an increased risk of multiple adverse maternal and offspring outcomes including preterm delivery, perinatal mortality, small for gestational age, congenital anomalies, and gestational diabetes. ART also is associated with differential offspring methylation of CpGs regulating known imprint control regions (ICRs). Prospective cohort study of mothers in the Newborn Epigenetic Study (NEST) cohort who answered questions concerning ART for the index pregnancy. Mothers exposed to clomifene without ART (embryo transfer (ET) or intra-cytoplasmatic sperm injection (ICSI)) measured 22 (N = 303 non-exposed) mothers reporting use of ART (ET or ICSI) numbered 27 (with N = 516 non-exposed mothers to either ART or clomifene). Methylation of 48 CpG sites in 9 ICRs was measured by pyrosequencing in mixed-leukocytes cord blood. In male offspring ART was associated with hypomethylation of the PEG5 ICR (β95% CI) = 1.46–2.81, 0.12) and hypermethylation of the MEG3 ICR (β91% CI (3.21–0.17, 4.7)). In female offspring, ART was associated with hypomethylation of the IG2F ICR (β3.67–6.79, 0.55). The use of clomifene without reported ET/ICSI was associated with hypomethylation of the PLAG1/HYMA1 and NNAT ICRs (β8.38 (13.53, 1.63) and -5.25 (10.12, -0.38) respectively) in male offspring. As in most studies of ART, exposure prevalence is low, limiting statistical power and we are unable to interrogate how extant maternal biology may contribute to outcomes. Similar qualitative patterns among these ICRs have been associated with cardiovascular, metabolic and behavioral outcomes in children. These results suggest that the increased risk of cardiovascular, metabolic and behavioral disorders in the offspring of ART-conceived children may in part be due to altered methylation of ICRs. Additional work with whole-epigenomic interrogation and replication is warranted.

2517a Vaping Inhalation Consequences on Placental and Pup Weight to Determine the Health Effects of Exposure in Maternal Gestation
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With the increase in popularity of electronic cigarette (e-cig) use, especially the JUUL, there has been a corresponding increase of use during pregnancy. This increase is associated with the marketing strategy that e-cigs are safer than regular cigarettes, research has shown that e-cigs still carry significant health risks. Therefore, there is a critical need to determine the effects of vaping during pregnancy. This research aimed to demonstrate the consequences of vaping during gestation on placental and pup weight with the hypothesis that the average pup and placenta weight will be lower in the JUUL exposed rats. Pregnant dams were exposed 6 times between gestational day (GD) 10-19 through a whole-body plethysmograph with rapid fill for a single exposure. One puff was released every 120s for a total of 30 puffs to the dams. The vape juice was non-nicotinic menthol-flavored and contained 30% propylene glycol/60% vegetable glycerin. The mean chamber concentration was 3.49x106 #/cc with a count median diameter of 3.49 nm per particle. Dams were sacrificed 24 hours after the last exposure on GD 20. Average pup weight was lower in JUUL exposed dams (3.61±0.11g; n=5 litters; n=44 pups) compared to the sham-air control group (5.72±0.80 g; n=11 litters; n=81 pups). This was a 37% decrease, which is very significant. Placental weight was not different between the JUUL exposed dams and the control group (0.72±0.02 g and 0.87±0.03 g, respectively). Placental efficiency, which is a ratio of pup weight divided by placental weight, was lower in the control group (5.1±0.16 vs. 7.10±0.26 respectively). This is a significant decrease of 28%. There was also an average increase in reabsorption sites within the JUUL exposed dams compared to the control (1.33±0.53 and 0.67±0.67 respectively). A reabsorption site is a dark-colored attachment site within the uterus. At this time, we conclude that the use of e-cigs during pregnancy has adverse maternal and fetal health effects such as significantly decreased birth weights and an increase of reabsorption sites within the uterus. Support: NIH ES015022 (TRN).

2518 Deep Learning-Powered Drug-Induced Liver Injury Prediction Using Model-Level Representation
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Drug-induced liver injury (DILI) is the most frequently reported single cause of safety-related withdrawal of marketed drugs. It is essential to identify drugs with DILI potential at the early stages of drug development. In this study, we describe a deep learning-powered DILI prediction model created by combining model-level representation generated by conventional machine learning (ML) algorithms with a deep learning framework. We conducted a comprehensive evaluation of the proposed DeepDILI model performance by posing several critical questions: (1) could the DILI potential of newly approved drugs be predicted by accurate knowledge of early approved ones? (2) is model-level representation more informative than molecule-based representation for DILI prediction? (3) could improved model explainability be established [where explainability refers to the ability of the parameters to justify the results]? For question 1, we developed the DeepDILI model using drugs approved before 1997 to predict the DILI potential of those approved thereafter. As a result, the DeepDILI model outperformed the five conventional machine learning algorithms and two state-of-the-art ensemble methods with a Matthews correlation coefficient (MCC) value of 0.342. For question 2, we demonstrated that the DeepDILI model’s performance was significantly improved compared with deep neural networks (DNNs) based on molecule-based representation. For question 3, we found 21 chemical descriptors that were enriched, suggesting a strong association with DILI outcome. Furthermore, we found that the DeepDILI model has more discrimination power to identify the DILI potential of drugs belonging to the WHO Therapeutic Category of ‘Alimentary tract and metabolism’. Finally, the DeepDILI model was applied to the recent real-world problem of predicting any DILI concern for potential COVID19 treatments from repositioning drug candidates. The interpretable model we have developed provides the opportunity to evaluate DILI causality of features (such as chemical descriptors) as well as reliability in predicting DILI for future drug candidates based on past knowledge. Altogether, the developed DeepDILI model could serve as a promising tool for screening for DILI risk of compounds in the preclinical setting.

2519 Expression and Localization of TRP Receptors Activated by E-cigarette (EC) Flavor Chemicals in Human Embryos
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Many EC aerosols contain high concentrations of flavor chemicals (e.g., menthol and vanillin), which can stimulate transient receptor potential (TRP) channels. We are testing the hypothesis that EC flavor chemicals adversely affect human embryos by activating TRP receptors. We mined the expression of TRP receptors in early stages of human development using a publicly available single cell RNA-seq (scRNA-seq) dataset containing mature oocytes, zygotes, 2-cells, 4-cells, 8-cells, morulae, late blastocysts, and human embryonic stem cells (hESCs), and then used immunocytochemistry (ICC) to confirm expression at specific location in hESCs. TRPV1, which is activated by menthol, cinnamaldehyde, and benzaldehyde, was abundant through the 4-cell stage. TRPV1 was present through the 8-cell stage and expressed in some morulae and blastocysts. Ethanol, which is used in some EC products, is a TRPV1 agonist. TRPV3, TRPV4, and TRPV6 were expressed in oocytes, zygotes, 2 and 4-cell embryos. Vanillin, a primary agonist for TRPV family, is a frequently used EC flavor chemical. TRPV2 and TRPMS were present through the 4-cell stage, and TRPMS, which is activated by ethanol, was also expressed in 8-cell embryos. Low levels of TRPM4 were present during the early stages.
of development, while high levels of TRPM6 mRNA were detected in the 8-cell stage, morulae, and blastocysts. TRPM7 and TRPM8 were in all stages studied. TRPV1 was absent in blastocysts and hESCs, while TRPP3 was elevated in the 8-cell stage and morulae. Expression of TRPC6, TRPV1, and TRPM8 in hESCs was confirmed by ICC. In agreement with the mRNA data, TRPM8 displayed negligible expression in hESCs, while TRPV1 was present around the edge of the nuclei. Seventeen TRP receptors were expressed at various stages of human development. Some can be activated by flavor chemicals and reaction products (e.g., acrolein) inhaled by EC users. Pregnant women are encouraged not to vape ECs until the effects of EC flavor chemicals on human embryos are fully understood.

2520 Reproducibility and Reliability of High-Throughput Transcriptomics for Chemical Safety Screening


High-Throughput Transcriptomics (HTTr) is a promising method for safety screening and prioritization of environmental chemicals. Decreasing costs have made it feasible to profile all protein-coding genes across thousands of samples, allowing for broad evaluation of many target pathways and modes of action in a single screening assay. US EPA is developing HTTr methods to rapidly screen chemicals in vitro, and has currently analyzed over 1,000 chemicals in 3 biologically distinct cell culture systems. The resulting data can be used for both hazard prediction and potency estimation, thereby informing risk assessment and prioritizing chemicals for further study. Both the reliability and reproducibility of this screening platform are critical to the utility of HTTr in regulatory applications. In this work, we characterize the reproducibility and reliability of HTTr using reference samples and chemicals included in the TempO-seq platform. We demonstrate the reliability of the HTTr assay using 4 standard reference samples that were profiled in duplicate on each of 333 individual screening plates across four independent studies utilizing several variants of the TempO-seq platform. We then demonstrate the high reproducibility of our complete HTTr chemical screening workflow—which also utilizes acoustic liquid handling systems to rapidly automate chemical treatments—by profiling reference chemicals that were replicated on each screening plate. Based on these reference treatments, we characterize the reproducibility of transcriptomic perturbation observed at multiple levels, from individual probes to gene sets capturing the activity of known biological pathways and signatures. We also demonstrate the high correlation across replicates for both single concentration response profiles and for biological pathway altering concentrations (BPACs) inferred from multi-concentration curve-fitting models. Overall, our results demonstrate that the high reproducibility and reliability of our workflow has potential applicability to risk assessment and prioritization of chemicals in a tiered-testing strategy. These results also inform best practices for HTTr data analysis, including sample-level quality control, and optimal methods for concentration-response modeling. This abstract does not necessarily reflect US EPA policy.

2521 Comparative Toxicogenomics Database (CTD): Linking Chemicals, Genes, Phenotypes, Diseases, and Exposures to Fill in Knowledge Gaps for Environmental Health

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The public Comparative Toxicogenomics Database (CTD; http://ctdbase.org/) is an innovative digital resource that connects chemical, gene, phenotype, anatomy, disease, and exposure information to advance understanding about environmental health. Literature-based, manually curated interactions are integrated to create a sophisticated knowledgebase that harmonizes cross-species heterogeneous data for chemical exposures and their biological repercussions. CTD is updated each month with new content and includes information on over 13,700 chemicals, 51,160 genes, 5,600 phenotypes (non-disease), 880 anatomical terms, and 5,800 diseases from more than 600 comparative species, as well as 168,000 human exposure measurements. Users enter CTD from any point-of-interest (e.g., chemical, gene, phenotype, anatomy, disease, etc.), and, because of seamless data integration, can discover novel connections that help fill in knowledge gaps for environmental health, including molecular initiating events (chemical-gene interactions), intermediate key events (phenotypes), adverse outcomes (disease), and population-level consequences (exposure studies and details) to help generate testable hypotheses. Additionally, CTD’s new anatomy webpages coalesce data for tissue-based exposures and chemical-phenotype toxicities, allowing users to survey information from an anatomical perspective, including pregnancy-related terms for early life exposure events. Examples are provided highlighting some of CTD’s numerous applications for discovery and insight into molecular mechanistic pathways, systems toxicology, exposures, and health outcomes related to environmental chemicals, such as ambient air pollutants, pesticides, polyfluoralky substances, and e-cigarette compounds.

2522 Pharmacophore, Ligand-Based, and Molecular Modeling Approach to Predict Novel Therapeutic Targets in Breast Cancer for Resveratrol

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GLOBOCAN 2018 database global statistics showed that breast (11.6%), prostate (7.1%), and colorectal cancer (6.1%) constitute the three most common types of cancer accounting for the majority of the incidence globally among women. Previous studies showed that resveratrol (Rsv) is known to act as an adjuvant for malignancies in the breast. Rsv and pyrazole-oxindole conjugates targeting tubulin polymerization are known to act as new anticancer agents. In this study, we predicted the novel breast cancer (bc) targets for cis and trans forms of Rsv using the pharmacophore and ligand-based approach. We have developed and tested the three-dimensional structure file for cis (CID: 48910) and trans-Rsv (CID: 445154) from the Pubchem database. Potential targets for cis and trans-Rsv were predicted using PharmMapper and SwissTargetPrediction servers. GeneCards and Online Mendelian Inheritance in Man (OMIM) were used for the collection of bc genes. Protein-protein interactions were performed using the STRING followed by gene ontology (GO) analysis using Database for Annotation, Visualization and Integrated Discovery (DAVID) and GO (http://geneontology.org/) database. We identified 100 protein targets and 10 bc genes for cis and trans-Rsv. Further, the interactions of these targets with cis and trans-Rsv were analyzed using a molecular modeling approach followed by the construction of cis-Rsv-bc and trans-Rsv-bc networks. Results from GO enrichment analysis showed that corticosterone 18-monooxygenase, prostaglandin-endoperoxide synthase, steroid 11-beta-monooxygenase, limonene monooxygenase, (S)-limonene 7-monooxygenase activity as top pathways enriched at a p-value <0.01. The identified significant targets, pathways, and functional annotations may be useful in further studies on elucidating the role of cis, trans-Rsv and other anti cancer agents such as pyrazole-oxindole conjugates in bc.

2523 DeePrescribing: A Deep Learning Framework for Predicting Drug-Drug Interactions

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Drug-drug interactions (DDIs) are unwanted side effects resulting from the concurrent consumption of two or more drugs, leading to morbidity and mortality. DDI accounts for approximately 30% of all adverse drug events and causes nearly 770,000 deaths worldwide. However, the performance of publicly available wet-lab based DDI identification strategies and in silico approaches are still suboptimal. More effective methods are still urgently needed. Here, we proposed a DeePrescribing, a deep learning framework for predicting drug-drug interactions and their clinical outcomes. Specifically, we developed a DDI-based ontology consisting of 585,649 DDIs and their clinical outcomes based on the MedDRA hierarchy and DDI information from the Drugbank database. Then, we proposed Composite Similarity Matrix (CSM), which is a novel descriptor to describe each DDI based on chemical similarities between target compound and positive compounds tested in different biological experiments by deriving the high-level representation from S-layer autoencoder. Finally, we employed an adaptive multiple label deep learning model for predicting clinical outcomes of DDIs. The proposed DeePrescribing achieved high performance with mean ACC (0.92), macro-precision (0.72), macro -recall (0.9), macro F-score (0.8), micro-precision (0.74), micro-recall (0.92), and micro F-score (0.82), which outperformed the state-of-the-art (SOTA) published DDI prediction models. Furthermore, the proposed DeePrescribing showcased the potential DDIs identification of COVID19 re-purposing candidates and QT prolongation caused by DDIs. In summary, the presented DeePrescribing is a promising tool and could be used to monitor potential DDIs during the pre-clinical phase before human testing in clinical phases.
Cellulose nanofibrils (CNF) were reported to decrease fat absorption and glucose release, suggesting their potential application as food additives or dietary supplements. However, the effects of CNF intake for an extended period of time remain unknown. The objective of the present study was to examine if CNF affected the gut microbiome and associated metabolic pathways. In the first study, male C57BL/6 mice were fed with a Western diet ad libitum and exposed to CNF for one month at a physiologically relevant dose (30 mg/kg) by gavage. The 16S RNA sequencing analysis of fecal samples indicated the gut microbiome communities were well separated for the unweighted Unifrac of β-diversity, but not for weighted Unifrac. Analysis of the α-diversity indexes suggested that the genetic richness was not significantly different among the groups. Further taxonomy profiling using LEfSe suggested that CNF treatment induced substantial gut microbiome shifts compared to either the water (WW) or cellulose (WC) at the same dose. PICRUSt analysis of functional metagenomics identified more than 50 pathways that might be altered following CNF ingestion compared to either the WW or WC treatment. Due to altered fatty acid biosynthesis, and starch, sucrose and fructose metabolism in CNF treated mice, a six-month chronic study using NODEF mice was subsequently conducted to investigate alterations in other physiological and behavioral endpoints. Bodyweight and glucose levels were taken weekly, behavioral studies were conducted every two months, along with fecal sample collection and glucose and insulin tolerance tests. Additionally, various organs were collected during necropsy for future processing. No major significant differences were seen in the weekly body weight, glucose levels or tolerance tests, behavioral studies, or organ weights. Collectively, our studies provide critical findings regarding the safety of long-term CNF consumption associated with the ecology of the gut microbiome and energy metabolism. Supported by the USDA National Institute of Food and Agriculture [grant no. 2016-67021-24994/project accession no. 1009090], and in part by NIH [grant no. R41DK121553].
For centuries, enzymes have been used to enhance food production due to their ability to accelerate biochemical reactions. *alpha*-Amylases (EC 3.2.1.1) act on polysaccharides to catalyze the hydrolysis of 1,4-alpha-D-glucosidic linkages and are affirmed as Generally Recognized As Safe as direct human food ingredients when obtained from a non-pathogenic and non-toxicogenic strain of *Bacillus stearothermophilus* (now *Geobacillus stearothermophilus*). An in silico analysis was conducted on a *G. stearothermophilus* strain ASM366767v1 (Genbank Accession Number GCA_003667675.1), the reference genome for *G. stearothermophilus* listed by the National Center for Biotechnology Information (NCBI), to determine whether the strain is pathogenic or toxicogenic. Annotation of the whole genome of *G. stearothermophilus* ASM366767v1 was conducted using PATRIC (v.3.6.7). The Basic Local Alignment Search Tool (BLAST) program maintained by the NCBI was used to conduct sequence alignment queries of the 3,468 coding regions from *G. stearothermophilus* ASM366767v1 against 7,235 downloaded peptide sequences of animal venom proteins and protein toxins obtained from a curated database maintained by UniProt. A sequence alignment of ≥40% identity, an E-value of <0.001, and a bit score of >50 were used as criteria for significant alignment to a known toxin. Using these criteria, no genes encoding for known toxins were identified. Sequence homology searches of the annotated genome were also conducted against manually curated databases of putative virulence genes (PATRIC, VFDB, and Victors). Four putative virulence factors were identified with query or subject coverage and percent identities ≥80%. BLAST searches of the protein sequences of these virulence factors against non-redundant sequences in the GenBank database revealed that these genes are commonly expressed in other strains of *G. stearothermophilus* and the *Geobacillus* species in general. Furthermore, *G. stearothermophilus* ASM366767v1 was predicted to be non-pathogenic to humans using PathogenFinder 1.1 (0.215% probability), and, following a search of the scientific literature, no *Geobacillus* species have been reported to be pathogenic. The results of the in silico analyses of *G. stearothermophilus* strain ASM366767v1 suggest that the strain is non-pathogenic and non-toxicogenic, and therefore, would be a suitable source of *alpha*-amyrase for use in food.

**Using a High-Throughput Screening Method to Pinpoint Genetic Mechanisms That Lead to Individual Susceptibility Differences in a Genetically Diverse Zebrafish Model**

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Differences in individual susceptibility to chemical exposures have broad implications in environmental health sciences and toxicology. Understanding the genetic mechanisms behind individual susceptibility differences is key to protecting vulnerable populations. However, elucidating gene-environment interactions (GxE) causing these differences presents daunting challenges in human epidemiological settings. Therefore, we leveraged high-throughput screening data from genetically diverse zebrafish to find evidence of GxE eliciting differential susceptibility to developmental malformation after exposure to the insecticide, Abamectin. We used a combined bioinformatic and experimental approach to probe the genetic mechanisms underlying why the morphological defects differed across the population. We generated whole-genome sequencing data to compare abamectin treated morphologically normal fish to phenotypically “affected” fish. This genome-wide analysis highlighted a region upstream of sox7 associated with differential response. Deep reanalysis identified an indel and several SNPs. Using RT-PCR, significant differences in sox7 expression were found between the affected and unaffected fish. Further analysis was conducted to predict differences in transcription factor binding sites between individuals with different sequences using Matbase. The analysis revealed many transcription factors that were predicted to bind differentially between the affected or unaffected fish. To validate the analysis, a gene-expression experiment where the sequences that were associated with different outcomes were cloned upstream of sox7 in an expression vector and then transfected into MCF-7 cells. Preliminary data shows significant expression differences in the presence or absence of the indel discovered upstream of sox7. Collectively, we demonstrated a method to identify chemicals that elicit high variation in individual susceptibility due to gene-environment interactions and probe to elucidate the causes of these effects.
To extend the capability of standard differential gene expression and pathway analysis, we have deployed a novel approach for characterizing mechanisms of action (MOA) of toxicological chemicals that involves network analysis and 3D visualization. Network analysis applies a rank-based statistical approach to calculate similarity between a query expression signature and signatures derived from other chemical/perturbagen transcriptomic experiments, enabling MOA predictions and transcriptomics read-across. As a case study, we evaluated liver TempO-Seq S1500+ transcriptomic data from rats exposed to the common environmental pollutant, perfluorooctanoic acid (PFOA). We first compared PFOA expression signature to those of 19 other chemicals assayed simultaneously. Network analysis results indicated PFOA bears mechanistic similarity with the peroxisome proliferator-activated receptor (PPAR) agonists fenofibrate, di(2-ethylhexyl)-phthalate, and triclosan; and the pregnane-X receptor activator tris(1-chloro-2-propyl)-phosphate. We then queried a collection of 10,363 highly curated rat gene expression signatures compiled using data from public sources such as TGGATES and DrugMatrix, which included varied perturbagens, organ/tissue systems, exposure durations, and concentrations. The PFOA query signatures were near a cluster of curated database signatures for fenofibrate, DEHP, and other chemicals that are known to activate PPARs. Notably, dose-dependence could be observed in the spatial positioning of the PFOA signatures for the eight doses in the study, with the signatures for increasing doses of PFOA being progressively closer to the database signatures of the PPAR-activating chemicals. The findings verify the hypothesis for primary MOA of PFOA and further suggest roles for less well-established MOAs of PFOA. Our results serve as a proof-of-concept for characterizing the MOAs of chemical toxicansts and facilitates comprehensive synthesis of transcriptomics data using novel bioinformatics inference. Interactive 3D visualization included in network analysis makes transcriptomics read-across easy and time-efficient.
High content imaging represents an emerging set of methods to assess total cell response to pharmaceuticals and environmental toxicants in a high-throughput manner. Here, our goal was to apply these methods to derive a chemical’s morphometric phenotypic “fingerprint”, classifying mode of action of per- and polyfluoroalkyl substances (PFAS) in a dose-dependent fashion. MCF10A breast epithelial cells were first exposed to 4 doses of histone deacetylase inhibitors of known molecular structure and function and analyzed via CellPainting, a high-content imaging assay, to optimize and validate our computational methods. We also explored MCF10A cells to 4 doses, ranging from 25nM to 25µM, of perfluoroalkyl substances PFDA, PFNA, PFOA, and the novel PFOA derivative “GenX”. Given the prospect of PFAS epigenetically reprogramming mammary development and promoting breast cancer, we analyzed features from the Hoechst 33442 stained nuclear channel. Following automated microscope image processing using CellProfiler, 240 nuclear features were calculated for over 42,000 cells. We used generalized linear models to rank each feature for its chemical specific significance and dose-dependent directionality, visualized through heatmaps and matrix plots. Given our method’s selectivity in identifying structure-bioactivity relationships, PFOA and its branched ether derivative GenX had low feature correlation (r = 0.43). This indicates contrasting bioactivities and suggests novel and distinct molecular targets of action for GenX, a poorly characterized toxicant recently introduced into the consumable technical and environmental environment. Further, this method effectively differentiated branched and linear PFAS, clustering the 8 and 9 carbon PFOA and PFNA with high feature rank correlation (r = 0.70). Heatmaps and unbiased clustering of feature rank and directionality demonstrated distinct morphometric fingerprints for each toxicant, identifying feature clusters with distinguishable structure-activity relationships, modes of action, and dose-dependent behaviors. We identified cellular morphological targets of PFAS previously unexplored with traditional single-cell and high-throughput techniques, potentially identifying new hallmarks of adverse cell response to these environmental perturbations.

Physiologically-based toxicokinetic (PBTK) models aid health risk assessment by incorporating in vivo and in vitro toxicokinetic data. We have recently shown that BPS, the second most abundant bisphenol, like BPA, can cross the placental barrier and disrupt placental function. Differences in physicochemical properties and toxicokinetics between BPA and other bisphenols prevent direct extrapolation of existing BPA PBTK models to BPS. We aimed to develop pregnancy-specific PBTK (p-PBTK) models for BPA and BPS. Three paired maternal and fetal pregnancy datasets of total, unconjugated, and conjugated BPA and BPS plasma concentrations from three independent studies in pregnant sheep were used for model calibration. The nine-compartment (maternal blood, liver, kidney, fat, placenta and rest of body, and fetal liver, blood and rest of body) models, which adhered to the WHO’s PBTK model evaluation criteria for use in risk assessment, simulated maternal and fetal BPA and BPS experimental data within one standard deviation for the majority of experimental data points, highlighting the robustness of both models. Dosing regimen was extrapolated to daily maternal exposure to BPA or BPS at the tolerable daily intake noted over a 2-week period to examine long-term fetal exposure. These simulations showed fetal accumulation of both bisphenols. BPS cleared slower from the fetal compartment than BPA. The steady-state approximation following this dosing strategy achieved a fetal bisphenol levels observed in cord blood from human biomonitoring studies. A global sensitivity analysis for both fetal kinetic and feto-placental transfer parameters was performed and revealed that feto-placental transfer and fetal conjugation and deconjugation parameters are the main drivers of fetal plasma bisphenol concentration variability. In contrast, the rest of body partition coefficient for both unconjugated BPA and BPS, minimally contributed to this variability, likely due to quick metabolism of unconjugated bisphenols. These models advance our understanding of bisphenolic compound toxicokinetics during pregnancy, provide the backbone for the development of accurate p-PBTK models with greater translational relevance, and can be used as a quantitative comparison tool in future p-PBTK models for related chemicals. 

Modeling of Bisphenols: Pregnancy-Specific Physiologically Based Toxicokinetic Models for BPA and BPS

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Physiologically-based toxicokinetic (PBTK) models aid health risk assessment by incorporating in vivo and in vitro toxicokinetic data. We have recently shown that BPS, the second most abundant bisphenol, like BPA, can cross the placental barrier and disrupt placental function. Differences in physicochemical properties and toxicokinetics between BPA and other bisphenols prevent direct extrapolation of existing BPA PBTK models to BPS. We aimed to develop pregnancy-specific PBTK (p-PBTK) models for BPA and BPS. Three paired maternal and fetal pregnancy datasets of total, unconjugated, and conjugated BPA and BPS plasma concentrations from three independent studies in pregnant sheep were used for model calibration. The nine-compartment (maternal blood, liver, kidney, fat, placenta and rest of body, and fetal liver, blood and rest of body) models, which adhered to the WHO’s PBTK model evaluation criteria for use in risk assessment, simulated maternal and fetal BPA and BPS experimental data within one standard deviation for the majority of experimental data points, highlighting the robustness of both models. Dosing regimen was extrapolated to daily maternal exposure to BPA or BPS at the tolerable daily intake noted over a 2-week period to examine long-term fetal exposure. These simulations showed fetal accumulation of both bisphenols. BPS cleared slower from the fetal compartment than BPA. The steady-state approximation following this dosing strategy achieved a fetal bisphenol levels observed in cord blood from human biomonitoring studies. A global sensitivity analysis for both fetal kinetic and feto-placental transfer parameters was performed and revealed that feto-placental transfer and fetal conjugation and deconjugation parameters are the main drivers of fetal plasma bisphenol concentration variability. In contrast, the rest of body partition coefficient for both unconjugated BPA and BPS, minimally contributed to this variability, likely due to quick metabolism of unconjugated bisphenols. These models advance our understanding of bisphenolic compound toxicokinetics during pregnancy, provide the backbone for the development of accurate p-PBTK models with greater translational relevance, and can be used as a quantitative comparison tool in future p-PBTK models for related chemicals. 

Development of a Physiologically Based Pharmacokinetic (PBPK) Model for Meloxicam in Broiler Chickens and Laying Hens

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Meloxicam is a non-steroidal anti-inflammatory drug (NSAID) commonly used in food-producing animals, including chickens. It was the drug that received the highest number of calls in the Call Centers of the Food Animal Residue Avoidance Databank (FARAD) from July 2019 to June 2020. This study aimed to develop a physiologically based pharmacokinetic (PBPK) model for meloxicam in broiler chickens and laying hens to facilitate development of a withdrawal interval. The PBPK model structure for broiler chickens included five compartments, including muscle, liver, kidney, fat, and rest of body tissue compartments, while additional compartments of ovary and egg were included for laying hens. Physiological parameters were collected from the literature. Partition coefficients were determined as the ratio of area under the curve between the tissue and plasma from a study in laying hens orally exposed to meloxicam at 1 mg/kg every 12 h for 20 administrations. The broiler chicken model was calibrated with plasma data from the FARAD Pharmacokinetic Database. Preliminary results showed that the model adequately simulated available pharmacokinetic data of meloxicam in plasma of broiler chickens with an estimated coefficient of determination (R²) of ~0.96. Model extrapolation from broiler chickens to laying hens and incorporation of parameter variability for Monte Carlo simulations to estimate meat and egg withdrawal intervals is still ongoing. Once completed, this model will provide a useful tool for safety assessment of meat and egg products treated with meloxicam, and will serve as a basis for extrapolation to other NSAID drugs and other poultry species to aid animal-derived food safety assessment.
The advent of high-throughput transcriptomic screening technologies has resulted in an array of available gene expression data associated with chemical treatment. From a regulatory perspective, data sets covering a large chemical space offer utility for the prediction of molecular initiating events associated with chemical exposure. Here, we integrate data from a large compendium of gene expression profiles with a catalog of chemical-target associations to train binary classifiers for predicting molecular initiating events (MIEs) from gene expression. To achieve this, we used ReFChemDB, a database of chemical-protein interactions, and LINCS CMAP data, a collection of gene expression profiles spanning multiple cell lines and chemical treatments. First, we linked perturbagens present in the LINCS gene expression data to DTXSIDs in RefChemDB. Next, we trained binary classifiers on MC7-trained gene expression profiles and chemical-target labels using six classification algorithms to identify optimal analysis parameters. To validate classifier accuracy, we used a variety of approaches, including multiple hold-out data sets, and permutation testing of “null” models. We identified 23 MIEs for which our training approach produced high performance classifiers that outperformed greater than 95% of permuted models. High performance classifiers were shown to corroborate ReFChemDB chemical-target linkages withheld from model training, demonstrating that predictive accuracy extends beyond the set of chemicals used in classifier training. To explore differences in MIE prediction as a function of cellular context, MC7 trained classifier accuracies were compared to orthologous classifiers trained on PC3 gene expression data, identifying classifiers that perform differently as a function of the cellular context of training data. This methodology can offer insight into prioritizing candidate perturbagens of interest for targeted screens, as well as selecting relevant cellular contexts for screening classes of candidate perturbagens. This abstract does not necessarily reflect US EPA policy.

New approach methodologies (NAMs) use *in vitro* and *in silico* models to predict toxicity based on a chemical’s bioactivity and molecular properties. Ideally, NAMs are developed and evaluated using well-characterized reference chemicals with defined activity against toxicity endpoints of interest. Structure-based information for chemicals is needed to properly define the domain of applicability for the NAM and to compare chemicals proposed for testing to reference chemicals used to develop and evaluate the NAM. The National Toxicity Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) developed the integrated Chemical Environment (ICE) Chemical Characterization tool to fill this need by allowing users to examine and compare chemicals’ structural and chemical properties, bioactivity, and functional use. Recent ICE tool updates allow users to compare these properties to better characterize their chemicals of interest drawn from the ICE chemical database of over 800,000 chemicals. Principal component analysis plots allow users to easily generate visual comparisons based on structural and physicochemical properties between groups of chemicals and to examine which properties drive the separation of chemicals within the space described by the principal components. ICE users can also determine if there are differences in bioactivity that distinguish their chemicals of interest. Additionally, information from the U.S. Environmental Protection Agency’s Chemical and Products Database has been recently added to ICE, allowing users to explore functional use categories and see how those relate to the available bioactivity data. This presentation will demonstrate the data users can obtain from the ICE Chemical Characterization tool with an emphasis on the new bioactivity and consumer product features. This project was funded with federal funds from the NIEHS and NIH under Contract No. HHSN273201500010C.

OPERA is a free and open-source/open-data suite of QSAR models providing predictions on toxicity endpoints and physicochemical, environmental fate, and ADME properties. All OPERA models are built on curated data and standardized for QSAR-ready chemical curations. OPERA follows the five OECD principles for QSAR modeling to provide scientifically valid, high accuracy models with minimal complexity that support mechanistic interpretation, when possible. The latest additions to OPERA include models for estrogenic activity, androgenic activity, and acute oral systemic toxicity developed through international collaborative modeling projects. Existing OPERA models are also updated regularly. Recently, the models predicting plasma protein binding and intrinsic hepatic clearance, two of the most important ADME parameters for *in vitro* to *in vivo* extrapolation, have been updated with the latest publicly available datasets to improve their predictivity and applicability domain coverage. Furthermore, models predicting physicochemical properties such as logKow, water solubility, and vapor pressure have been updated to account for highly investigated groups of chemicals such as polyfluorinated substances (PFAS). In addition to predictive models, OPERA provides a tool for standardizing chemical structures, an estimate of prediction accuracy, an assessment of applicability domain, and experimental values when available. Technical and performance details are described in OECD-compliant QSAR model reporting format (QMRF) reports. OPERA predictions are available through the EPA CompTox Chemicals Dashboard and the National Toxicology Program’s Integrated Chemical Environment. The OPERA application can also be downloaded from the NIEHS GitHub repository as a command-line or graphical user interface for Windows and Linux operating systems. This project was funded in whole or in part with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C. The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the US EPA.
2544 Bridging the Gap between In Vitro Assays and Real-World Patient Data with Novel Statistical Models

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Though animal-based testing regime has been the mainstay of the biological mechanisms of toxicity, its high cost and long timeframe are among other drawbacks. In vitro assays have the advantage of being high throughput and able to pinpoint specific biological processes, but are often criticized for the lack of validation with direct human evidence. Although performing specific clinical trials for the purpose of validating in vitro assays will only be possible on rare occasions, current development in collecting and utilizing real-world patient data has provided unique opportunities to corroborate results from in vitro assays using human evidence of drug toxicity. However, bridging the gap between in vitro assays and real-world human data requires novel statistical approaches. In this paper, we provide examples on progresses in this area, focusing on drug induced liver injury (DILI) using a wide range of in vitro assay data in triangulating evidence from FDA Adverse Event Reporting System (FAERS) database and National Center for Health Statistics (NHANES) surveys. We demonstrate that predictions of toxicity using in vitro assays can be validated with data from postmarket surveillance databases. As a case study, we investigated a predictive model for DILI using in vitro assays. This penalized logistic regression model has been built with high dimensional in vitro assay data (nuclear receptor activity, gene expression, and cellular functions) after dimensional reduction by filtering through adverse outcome pathway (AOP) networks. The linear predictor for DILI derived from this model is then used to fit a Poisson regression model for DILI reporting counts in the FAERS database with adjustment for drug specific baselines. It is shown that the increase of one in the linear predictor value leads to 30% higher DILI reporting counts over the baseline level, which is highly significant. This adjusted Poisson regression model is also highly flexible and can be used to correlate sex or age biased molecular pathways to differential reporting patterns in FAERS, thus enabling investigation of host factors at both molecular and postmarket surveillance level. The incorporation of NHANES and other sources further improve the reliability of the results. Further development of this approach can strengthen the utility of both in vitro assays and postmarket surveillance data.

2545 Trade-Off Predictivity and Explainability for ML-Powered Predictive Toxicology: An In-Depth Investigation with Tox21 Datasets

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Selecting a model in predictive toxicology often involves a trade-off between prediction performance and explainability: should we sacrifice the model performance to gain explainability, or vice versa? Here we present a comprehensive study to assess algorithm and feature influences on model performance in chemical toxicity research. We conducted over 5000 models for a Tox21 bioassay dataset of 65 assays and ~7600 compounds. Seven molecular representation methods were compared using several modeling approaches varying in complexity and explainability were employed to systematically investigate the impact of various factors on model performance and explainability. We demonstrated that endpoints dictated a model’s performance, regardless of the chosen modeling approach including deep learning and chemical features. Overall, more complex models such as (L5/SVM and Random Forest performed marginally better than simpler models such as linear regression and KNN in the presented Tox21 data analysis. Since a simpler model with acceptable performance often also is easy to interpret for the Tox21 dataset, it clearly was the preferred choice due to its better explainability. Given that each dataset had its own error structure both for dependent and independent variables, we strongly recommend it is important to conduct a systematic study with a broad range of model complexity and feature explainability to identify model balancing its predictivity and explainability.

2546 Variability in Organ-Level Effects in Repeat-Dose Animal Studies

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Building scientific confidence in the use of new approach methodologies (NAMs) in safety assessment may include performance comparison to in vivo study outcomes. This work defines the variability in organ-level effects and suggest qualitative and quantitative benchmarks for maximum NAM performance for prediction of organ-level effects in repeat dose studies of adult animals. Previous work suggests that the root residual mean square error (RMSE) for study-level lowest effect level (LEL) values (on a log$_{10}$mg/kg/day basis) approaches 0.5 log$_{10}$mg/kg/day. Observations of liver, kidney, stomach, spleen, thyroid and adrenal gland from the Toxicity Reference database (v2.0) were included. The percent of chemicals with concordant organ-level findings across replicate studies, and the variance in treatment-related organ LEL values, were estimated. The largest dataset available with >1 study by chemical included >500 chemicals. Replicate datasets were defined by chemical only, chemical and species, and chemical and study type to estimate concordance in observed organ-level effects (repeated presence/absence of weight, gross or histopathological changes) for a chemical. Total concordance (% chemicals with positive or negative agreement across replicates), depending on the organ and replicates defined, ranged from 39 - 88%, with slightly greater average within-species concordance. Organs associated with more negative chemicals (stomach, thyroid, adrenal) had slightly higher rates of concordance in this range. Multilinear regression modeling, using study type, species, administration method, dose number, dose spacing, substance purity, and study year as descriptors of LEL data by organ, was used to estimate total variance, mean square error (MSE), and the RMSE. With MSE used to indicate unexplained variance, results suggest study descriptors accounted for 52-69% of the total variance in organ-level LELs. A NAM would be unlikely to explain more than the variance explained by study level descriptors, or 70% of the variance in these data. The RMSE for these organ-level statistical models ranged 0.4 - 0.6 log$_{10}$mg/kg/day, suggesting organ-level variance in LEL values was similar to overall study LEL variance. Therefore, a good NAM might predict organ-level LELs within ± 1 log$_{10}$mg/kg/day. This work suggests thresholds on NAM accuracy for repeat dose, organ-level effects in adult animals. This abstract does not necessarily reflect US EPA policy.

2547 Measuring Stress Response Pathways in Transcriptomic Data


The Environmental Protection Agency (US EPA) has set a goal of eliminating animal testing by 2035, spearheading the implementation of high-throughput screening technologies (HTS) for informing hazard and risk. We are developing new approach methodologies (NAMs) for rapidly screening chemicals using high throughput transcriptomics (HTT) to quantify cellular pathway activities associated with molecular initiating events (MIEs) in adverse outcome pathways (AOPs). Here, we present our findings on a HTT-NAM to characterize the activity of chemicals by evaluating the transcriptional activity of stress response pathways (SRPs). First, we developed gene signatures of six canonical SRPs: DNA damage response (DDR), unfolded protein response (UPR), heat shock response/proteotoxic (HSR), response to hypoxia (HPX), metal-associated response (MTRL), and oxidative stress response (OSR). We first developed consensus signatures from the Molecular Signatures Database (MSigDB) using a frequency-based approach. Second, we identified 90 reference perturbagens (68chemicals and 20 genetic perturbations) from the Library of Integrated Network-based Signatures (LINCS) that induced specific SRPs by curating PubMed abstracts. Third, we used connectivity mapping to match 11,000 transcriptomic profiles for the 90 reference perturbagens from LINCS with gene signatures for SRPs. Resulting data indicate that 66chemicals within the 70 predicted chemical perturbagens illustrate SRP activation and may proceed through injury endpoints. UPR, HSR, HPX, and OSR were most accurately classified with 100% of predicted reference chemicals identified. The DDR consensus signature identified 92% of reference chemicals while the MTL consensus signature was only able to capture 50%. The most frequently correctly match cell lines were VCAP, HA1E, and MCF7 cells. These results indicate that SRPs act in discrete systems with measurable biological activity. In addition, we are investigating additional measures of stress associated cellular and study year as descriptors of LEL data by organ, was using ultimate SRP-linked cell fate. Further development of this approach may ultimately lead to a NAM with the potential to characterize stress pathway activity. The views expressed in this presentation are those of the author(s) and do not necessarily reflect the views or policies of the US EPA.
of scalable computational models to support new approach methodologies (NAMs) for chemical hazard identification. NAMs aim to improve predictive toxicology with less reliance on animal testing. Here a model was developed to identify chemicals connected to both ATRA pathway bioactivity and prenatal skeletal defects. Chemicals (n=375) from ToxRefDB and/or ToxCast altered skeletal phenotypes in prenatal developmental toxicity studies. Defects were organized into 10 skeletal phenotype regions of interest: cranial (neurocrania, orofacial, and viscerocrania); axial (thoracic cage, vertebral, and caudal); appendicular (stylopod, zeugopod, and autopod); and other non-specific skeletal structures. For each chemical, distribution of phenotype(s) was scored as a fraction of 1 for inclusion in ToxPi k-means clustering. The clustering identified trends in skeletal defects due to shared characteristics among chemicals. To complete the key model, we evaluated results from >8,070 chemicals in ToxCast/Tox21 across 13 in vitro assays for components in the retinoid system. Over 40 chemicals were identified for constructing data-driven models to link this in vitro data with skeletal adverse outcomes (AOs) in ToxRefDB and ToxCast. This abstract does not represent the views of the Agency.

2549 Bridging In Vitro DDE Obesogenicity Data with Epidemiologic Studies through the Use of Biological Modeling

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Current acceptable exposure levels are mostly based on animal models, which are costly, time-consuming, and may poorly predict adverse outcomes in humans. There is a need for alternative testing methods that are faster, cheaper, and provide human-relevant information. Our objective is to evaluate a method using human in vitro data and biological modeling to calculate an acceptable exposure level through a case study on p,p’ DDE obesogenicity due to early life exposure. To this end, we reviewed in vitro studies on p,p’ DDE and obesogenicity-related endpoints to select a point of departure (POD). We subsequently translated this POD expressed in terms of nominal concentration into a cellular level using a mass-balance model. This cellular concentration (target organ POD) was converted into an acceptable daily intake and associated in pregnancy with women using a pharmacokinetic model of gestation/lactation and accounting uncertainty factors. Finally, we compared the derived maternal plasma DDE level to median levels measured in epidemiological studies reporting associations between prenatal exposure and child obesity. For this study, we used a POD of 0.1 μM, representing the lowest observed adverse effect level (LOAEL) of downregulation of PLIN2 gene expression during mesenchymal stem cell differentiation (cellular concentration of 14.6 ng/g cells), which translated into a maternal plasma concentration of 42 ng/g lipids. Adjusting for uncertainty factors (3000), we derived an acceptable maternal daily intake (0.0012 ng/kg/d) and associated plasma concentration during pregnancy (0.014 ng/g lipids). Epidemiological studies reported median levels ranging from 1.1 ng/g lipids to 2.700 ng/g lipids. Overall, the plasma concentration derived from in vitro data was below the range of median levels measured in epidemiological studies. The use of a POD based on gene expression (early response) along with a high composite uncertainty factor may be too conservative.

2550 Dexter: A Semi-Automated Data Extraction Tool to Support Literature-Based Health Assessments

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Across the field of toxicology, the use of systematic review methods to maximize transparency and minimize bias in literature-based assessments is gaining momentum. Literature assessments are fit for purpose. Scoping reviews utilize systematic review methodologies to summarize research on broad topics in order to identify data rich and data poor areas to inform evidence-based decisions on further research or analysis; and are often accompanied by a systematic evidence map which categorizes and displays key concepts to illustrate the extent and types of evidence in an interactive visual format. Systematic reviews are used to identify, select, critically assess, synthesize data and integrate the evidence on a specific question to reach conclusions on human health hazards associated with exposure to xenobiotics. Conducting an assessment is time-consuming and resource intensive – often requiring more than 1,000 hours and $100,000 USD. The Division of the National Toxicology Program (DNTP) is developing and evaluating Dexter - a web-based data extraction tool designed to address the resource-intensive step of data extraction in literature-based assessments. Dexter was developed with a user interface that provides a flexible, semi-automated, and connected complex data in a hierarchical structure as publications may report multiple experiments with various exposures and doses and evaluate multiple endpoints. The usability of the tool within the DNTP workflow was evaluated to determine what efficiencies would be gained by using Dexter’s semi-automated data extraction workflow. Dexter was evaluated in both the manual extraction mode and the semi-automated extraction mode in the tool and results were compared with a gold standard dataset for time to complete data extraction and accuracy. Data extractions using the semi-automated mode showed considerable reduction in the average time for completion (47% shorter total time) with higher precision (semi-automated 93.2%; manual 92.5%) balanced by a trade-off in lower recall (semi-automated 83.8%; manual 91.8%). Dexter’s results offer promise for successfully integrating natural-language-processing methods into a tool that improves the data-extraction process of literature-based evaluations while maintaining quality and reproducibility.

2551 Construction of a AOP Network Related to Metabolism Disorders Induced by an Endocrine-Disrupting Chemical Mixture Using Artificial Intelligence and Systems Toxicology

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Metabolic disorders are among the main adverse health outcomes that have been associated with endocrine disrupting chemicals (EDCs). It is essential to have a better understanding of the mode of action of suspected EDCs, and the biological pathways that they may be perturbed to identify their real impact on the human population. The concept of Adverse outcome pathways (AOP) provides a practical organizing framework of perturbations at different levels of the biological organization by linking molecular initiating events (MIE) to an adverse outcome (AO) across several intermediate key events (KE). We investigated the applicability of an integrated systems toxicology approach to develop a AOP network related to metabolism disorders. First, a new tool called AOP-helpFinder was used to identify metabolic effects of EDCs. One method to discern the action of EDCs is a population-based or fingerprint methods to assign, classify or quantify newly tested/retrospective, reactionary and quantitative (i.e. LC50, IC50, etc.). Although endpoint-based methods such as biopivots, PFAEs, PCBs, between compounds. AOP-helpFinder combines text mining and graph theory to automatically screen abstracts from the PubMed database. The tool searched for co-occurring terms among two lists: one that contains the studied EDCs, and one with the biological effects (from the AOP-wiki database and from in house experts). This step allowed to decipher links between each EDC and biological events, and was followed by a manual curation to select the most relevant publications, whose reliability was assessed by a dedicated tool. Then, an AOP network was proposed, which was enriched by integration of databases information. The tool searched for in vitro DDE obesogenicity data with epidemiologic studies reporting associations between prenatal exposure and child obesity. For this study, we used a POD of 0.1 μM, representing the lowest observed adverse effect level (LOAEL) of downregulation of PLIN2 gene expression during mesenchymal stem cell differentiation (cellular concentration of 14.6 ng/g cells), which translated into a maternal plasma concentration of 42 ng/g lipids. Adjusting for uncertainty factors (3000), we derived an acceptable maternal daily intake (0.0012 ng/kg/d) and associated plasma concentration during pregnancy (0.014 ng/g lipids). Epidemiological studies reported median levels ranging from 1.1 ng/g lipids to 2.700 ng/g lipids. Overall, the plasma concentration derived from in vitro data was below the range of median levels measured in epidemiological studies. The use of a POD based on gene expression (early response) along with a high composite uncertainty factor may be too conservative.

2552 Atomic Contribution Mapping and Exploration with Reverse Fingerprinting (ACME-RF): Assigning Toxicological Endpoints to Chemical Structure at Atomic Resolution

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The tools and model systems (in silico/vitro/vivo) for assessing biological activity, are usually defined by endpoints and metrics that are determinate, retrospective with factorialization (i.e. IC50, EC50, ...,). Although it is useful from a structure-activity relationship perspective to use descriptor-based or fingerprint methods to assign, classify or quantify newly tested/created chemicals based on these analyses, these approaches provide limited or no information about molecular moieties that give rise to the endpoint. One method to discern molecular features that could be triggered by several EDCs was then proposed. These findings highlight the increasingly relevant use of computational tools in predictive toxicology.
duce the concept of atomic contribution mapping and exploration (ACME) using the RF framework in a newly designed interface for use in the Molecular Operating Environment. Using publicly available datasets we systematically explore three different ACME-RF examples. In the first example we demonstrate the rapid identification of a class of pyrethroid acaricide that is not-toxic to honeybees while still being toxic to the varroa mite using very basic insecticide-class information of 80 pyrethroids as inputs. In the second example we used the ToxCast NVS_NR_HER dataset (165/2645) to build a RF model that was used to identify the toximorph of HER-a that directly maps to known co-crystallized structures. In the final example we explore photostability half-lives (Blum, Kristin M. 2013) and identify photobleatable moieties of chemistry that lead to photodegradation, a key liability for drugs and pesticides. Using ACME-RF we identified and visualized moieties of the molecules that resulted in specific (I) apical endpoints across species (II) chemical-biological interactions and (III) photodegradation. The method can be used not only to identify toxic chemicals, but also to identify critical toximorphic fragments essential for both molecular discovery and de-risking. This abstract solely represents the views of the authors and not the view of the Agency.

2553 Substructure-Based Strategies Supporting Analog Selection for Structure-Activity Relationship (SAR) Toxicological Assessments

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SAR (Structure Activity Relationship) based read across is an important animal alternative method used to fill toxicological data gaps. The safety of a target compound or structure of interest (SOI) lacking toxicological data may be assessed using data for source compounds or analogs. One of the most challenging aspects of the technique involves identifying suitable analogs providing the best match in toxicological profiles used to read across to the safety of the SOI. Current methods rely on searching toxicological databases by structure using a quantitative similarity score generated by comparing molecular fingerprints using algorithms such as the Tanimoto equation. While this process may produce many analogs for consideration, they may not be appropriate for read across, displaying divergent toxicological profiles from the SOI. An alternative method for comparing pairs of chemical structures including target/analog pairs is the matched molecular pair (MMP) approach. An MMP is defined as a pair of compounds that differ only by a single localized structural change and is widely used in the area of medicinal chemistry to associate small changes in structure with changes in properties. Here we demonstrate the use of MMP analysis of target/analog pairs of compounds to identify structural changes linking the two that lead to small changes in reactivity, metabolism and physical chemical properties that are interpreted in the context of analog suitability. Structural changes are then binned into five different categories for aromatic and aliphatic MMPs, respectively. A strategy for searching open source toxicological databases (e.g. OECD Toolbox) for potential analogs is presented and applied and compared to analogs selected based on quantitative comparisons of molecular fingerprints.


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A diverse range of chemicals have been associated with behavioral and neu-rocognitive deficits following in vivo developmental exposure. However, fewer than 200 chemicals have been tested in EPA and/or OECD guideline developmental neurotoxicity (DNT) studies, leaving thousands of chemicals without information on their DNT hazard potential. To address this gap, a suite of DNT new approach methodologies (NAMs) has been proposed for screening and prioritization. One approach to building confidence in DNT NAMs involves performance evaluation using reference chemicals with evidence of DNT effect (“positive” compounds) and lack of DNT effect (“negative” compounds) following in vivo mammalian exposure. As positive chemicals were previously established, our aim was to curate a list of negative reference chemicals. These DNT reference chemicals could then estimate the sensitivity and specificity of the DNT NAMs. A set of 37 candidate negative chemicals were evaluated using the Abstract Sifter Excel-based application to identify relevant studies which were then reviewed by a panel of experts. To determine whether a study had sufficient scientific rigor for assessing in vivo DNT hazard, experts evaluated the experimental design and developmental outcomes using a series of pre-defined questions. After reviewing the body of evidence, the expert panel discussed each chemical and wrote narrative reviews summarizing the results. The panel determined that the level of evidence for 7/37 chemicals supported their use as negative DNT reference chemicals, whereas 6/37 were considered unsuitable based on evidence implicating direct DNT effects. The remaining 25/37 were cast as equivocal, with less confidence in their use as negative chemicals given potential caveats (e.g. in vivo neuroactivity, confounded clinical reports, and experimental design challenges). This reference chemical set may be customized to support performance evaluation of specific DNT NAMs, depending on the assay principle and key processes included. Further, this work suggests that additional approaches to DNT NAM performance evaluation may be required. This abstract does not reflect US EPA policy.

2555 Evaluation of In Vitro New Approach Methodologies for Developmental Neurotoxicity


Current developmental neurotoxicity (DNT) hazard assessment relies on in vivo testing that is resource intensive and lacks information on key cellular processes affected by chemical exposures. To address these limitations, DNT New Approach Methodologies (NAMs) are being developed and evaluated for their utility to inform DNT hazard. Here, we evaluated the combined performance of two DNT NAM technologies: the microelectrode array network formation assay (NFA) which uses primary rat cortical neurons to evaluate neuronal function, and high-content imaging (HCI) assays which use primary rat cortical neurons or human neural stem cell-derived cultures to evaluate proliferation, apoptosis, neurite outgrowth (NOG), and synaptogenesis. Combined, these assays include 57 endpoints when analyzed using the ToxCast Data Pipeline. Separate hierarchical clustering of the data set of 92 chemicals screened in the NFA and the HCI assays resulted in two distinct clusters of ‘high’ and ‘low’ activity. The ‘low’ activity cluster in both assay dendrograms included 10/10 reference DNT negatives, as well as 20/32 reference DNT positives in the HCI assay, and 14/32 positives in the NFA. Next, we evaluated if disruptions in proliferation, NOG, and/or synaptogenesis in the HCI assays corresponded with changes in network activity in the NFA. Chemicals that disrupted at least one HCl assay also generally decreased network activity (>15/17 down endpoints were hits). Given the diversity of responses covered by HCl, lack of differential patterns in the NFA, and high number of assay endpoints, we used random forest (RF) to reduce the total number of features used to identify DNT. An RF model using all 57 DNT NAM assay features and 42 DNT reference compounds resulted in 66.0% accuracy, 84% specificity and 71% sensitivity. The (14 of 57) most important DNT NAM features, including a subset of HCl and NFA endpoints, resulted in the highest model accuracy of 72.0%, with 100% specificity and 71% sensitivity. Collectively, this preliminary evaluation indicates that joint application of the NFA and HCI assays may perform better than either individual technology. A longer list of chemicals is currently being screened and will provide additional data to develop accurate and efficient models for DNT hazard assessment. This abstract does not reflect US EPA policy.

2556 Binding Site Complementarity and Screening Data Analysis to Identify Off-Target Propensity: A Case Study from Global Kinase Panel Screening

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Drug Safety Research and Evaluation, Pharmaceutical Research Division, Takeda Pharmaceutical Company Limited, Fujiwara 251-8555, JapanDrug molecules binding to off-targets can cause unwanted side effects and is one of the major drivers for the attrition of molecules. Identifying off-target binding propensity at early stages when the project starts enables us to prioritize the project from a toxicity perspective along with target safety review or propose counter screening to avoid the off-target effects. Off-target binding of molecules is more likely if the primary protein target has a significantly similar active site (both sequence and shape) with other proteins. To identify those off-targets in the early stages of the project we used 3Decision’s (https://www.discngine.com/3decision) algorithm that can identify the 3D shape of similar active sites. The algorithm can map the shape of the binding site(s) in case of multiple solved structures and use that information to retrieve protein targets having similarity in shape. The hits generated were then aligned and visually analyzed; finally, the possible off-targets were recommended. We report here on a prototype kinase, where we used the algorithm to recommend potential off-target kinases that can be inhibited by the designed
In support of non-animal alternative methods, it is important to understand mode of action of chemicals involved with the liver when target organ toxicity is addressed. For example, drug-induced liver injury remains a challenge, posing uncertainties when translating knowledge from the pre-clinical stages to human use cases. Whilst numerous efforts to develop prediction methods have been attempted, including in silico approaches such as QSAR, in general the success has been somewhat limited. To this end, through the Cosmetics Europe Mode-Of-Action (MoOA) Ontology project we were able to develop liver toxicity knowledge into ontology-driven representations of structural rules leading with insights on MoOA or Adverse Outcome Pathways (AOPs). By transforming published liver toxicity structural alerts into a chemotype hierarchy by encoding structural knowledge into CSRML (Chemical Structure Reaction Mark-up Language), we were able to implement liver toxicity knowledge into ontology-driven representations of structural rules leading with insights on MoOA or Adverse Outcome Pathways (AOPs). A combination of public datasets and in-house measurements are used to build thousands of models based on systematic hyperparameter searches. The most accurate models are selected for further development within the platform. Due to the small dataset size, the best models are generally found to be random forests built using smaller chemical descriptor set features. Additionally, classification models outperform regression models in general. The phase three of developing a DILI prediction platform is to identify how best to combine all of the individual assays and models. Three experiments are proposed: 1) Use the data from the ten main assays (plus additional predicted values) as features in a machine learning model to predict a human DILI label. 2) Apply empirical knowledge of DILI to develop a predictive, mechanistic model that uses the assays as input. 3) Develop a QSAR model of DILI. Experiments 1 and 2 are anticipated to have a higher DILI prediction accuracy than experiment 3. Future directions include applying an active learning loop in order to select new compounds that will have the greatest effect on improving the accuracy of the platform with a minimum number of new experiments.

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Initiatives that promote Findable, Accessible, Interoperable, and Reusable (FAIR) data principles are becoming a key criteria of public research funding agencies. FAIR principles enable new knowledge to be derived from existing data with minimal additional investment in data generation. The Michigan State University (MSU) Superfund Research Center (SRC) has produced vast amounts of data characterizing the mechanisms and impact of exposure to arylic hydrocarbon receptor (AhR) ligands. To promote their FAIRness, we have developed FAIRTox, an open-source web-based data exploration, visualization, and analysis application for toxicogenomic datasets. FAIRTox is built using a R Shiny framework, chosen due to its wide use as a tool for omics data analysis, and the availability of other R packages for enrichment and multidimensional analyses such as principal component analysis (PCA). Unprocessed and analyzed datasets are stored in a SQL relational database enabling querying through a user-friendly interface. Metadata filters allow users to visualize and compare gene expression responses to various experimental factors such as zeitgeber time, dose, and duration of exposure to various environmental contaminants. Enrichment and advanced analysis features also facilitate the integration of datasets furthering the development of novel hypotheses. In addition, the implementation of FAIRTox makes publicly available MSU SRC toxicogenomic data more accessible to researchers without transcriptomic databases.
expertise. FAIRTox is implemented as a Docker container ensuring portability and reproducibility for groups looking to execute their own local version. Ultimately, the goal of FAIRTox is to improve data sharing, reuse, and reproducibility through an intuitive interface that serves bioinformaticians and novices alike. Funded by the Superfund Research Program P42ES04919.

2561 High-Throughput Screening to Predict hERG Inhibition

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The human ether-a-go-go related gene (hERG) potassium channel, a member of voltage-gated potassium channels, plays a pivotal role in cardiac rhythm and function, essentially in the regulation of heart beat. Inhibition of hERG channels can lead to a prolongation of the QT interval, which, in the worst case, triggers torsade de pointes arrhythmia and can progress to ventricular fibrillation and sudden death. Environmental toxicants have the potential to contribute to the pathophysiologic of complex diseases, but the underlying mechanisms remain obscure and to date, more than 100,000 chemicals have been introduced into commerce without toxicological testing. An evaluation of the effect of environmental chemicals on hERG channel function can help inform the potential public health risks of these compounds. To assess the effect of environmental chemicals on hERG channels, the US federal Tox21 program has screened a collection of 9667 chemicals using a cell-based thallium influx assay in a quantitative high throughput screening (qHTS) format. The chemical results in the hERG qHTS assay were characterized using a set of 1D/2D molecular descriptors and physicochemical properties, Self-Organizing Maps (SOM) and hierarchical clustering. Statistical machine learning approaches were used to build potential structure-activity relationships (QSARs) of models to predict the probability of a chemical to inhibit hERG in this thallium flux assay, applying both classification and regression techniques. Models were compared with existing QSAR hERG models and dataset. The evaluation of performance criteria of generated models revealed that Random Forest model outperforms other models and demonstrated 0.89 cross-validated, 0.996 test set accuracy and equivalent performance against external test sets. This tiered clustering and predictive modeling approach facilitates detection of environmental chemicals that merit more extensive evaluation for cardio toxicity and provides useful structural information that could be applied to predicting the potential for new chemical entities to inhibit hERG.

2562 Information Retrieval and Network Analysis in ComptoxAI: A Data Infrastructure for AI Research in Computational Toxicology


Previously, we announced the development of ComptoxAI - an ongoing body of research focused on building a comprehensive data infrastructure for artificial intelligence applications in computational toxicology. Here, we showcase new functionality and scientific applications of ComptoxAI in two areas: (1) information retrieval (IR) in the context of cheminformatics workflows, and (2) analysis based on network data science that comprise ComptoxAI’s core graph database. ComptoxAI now supports retrieval and normalization of chemical properties, external database identifiers, and network relationships (e.g., genes or pathways modulated by exposure to specific chemicals) via a web API, enabling programmatic access and integration into arbitrary cheminformatics workflows. The data infrastructure in ComptoxAI is now being integrated into data analysis workflows at the Center of Excellence in Environmental Toxicology Exposure Biology Informatics Core at Penn, and are openly available for public use and expansion. Future additions in these areas include question answering features and responsive graphical interfaces to the data on top of the low-level programmatic API access. ComptoxAI also supports artificial intelligence research through automated machine learning, graph machine learning, and ontology inference. Rather than a relational database (where data elements consist of rows in tables), ComptoxAI uses a graph database format that is better able to represent and study the complex relationships that link entities of a wide variety of types (e.g., chemicals, genes, clinical outcomes, and others). By computing eigenvector centrality on each of the entities in the database and then aggregating based on ontological classes, we show that Adverse Outcome Pathway (AOP) data play crucial roles in linking semantically related regions of the graph. Specifically, when AOP data are excluded, both the average shortest path between any two entities and the overall diameter of the database increase dramatically. We expand on this finding to show that the AOP data act as critical “hub” points in the graph, providing important translational contexts between chemical exposures and specific diseases. These developments help to legitimize the importance of the AOP framework, as well as to prioritize future development of ComptoxAI and similar computational toxicology projects. Supported by P03-ES013508 to TMP and T32-ES019851 to JDR.

2563 Combining In Silico Structure-Based Methods and Enrichment Analysis to Increase Confidence in HTS Results: Application to Tox21_TRHR Assay

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Despite progress in applying high-throughput screening (HTS) technologies to toxicology, exemplified by the Tox21 and ToxCast programs, the challenge of relating biochemical outputs to molecular initiating events (MIEs) and adverse outcome pathways (AOPs) remains challenging, particularly when the relationship between the biochemical output and MIE is indirect. To increase confidence in HTS results for hazard evaluation, a structure-based data enrichment and knowledge contextualization workflow was developed to identify likely false positives and discriminate true receptor binders. The tiered approach was evaluated in vitro data for the Thyrotropin-Release Hormone Receptor (TRHR), an MIE in the thyroid hormone AOP. The tiered approach: 1) identifies structure-activity patterns using chemotype-enrichment (most a bio/day), expanding the inference to 122 assay inter-referenced structural modulators, benzodiazepines and neuropeptides. The presented tiered workflow is grounded by physical determinants directly related to relevant signals in experimental results, thereby increasing the value of in vitro data for chemical prioritization. The workflow can be generalized to other in vitro biochemical assays and can be used as a complementary structure-based and data enrichment analysis can increase confidence in HTS results and define a scope of prediction for risk assessment. This abstract does not reflect US EPA policy.

2564 High-Throughput Toxicokinetics Exposure Inference for Environmental Chemicals

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There is a need to rapidly assess potential risk for chemicals present in our environment, but the necessary toxicokinetics (TK) data are unavailable for most chemicals. High Throughput TK (HTTK) methods provide the opportunity to characterize large numbers of chemicals by combining in vitro measurements and in silico predictions of chemical-specific TK properties with generic TK models. The US EPA provides HTTK methods through the freely available R package “httk”. The U.S. CDC conducts the National Health and Nutrition Examination Survey (NHANES), which provides biometric and chemical exposure monitoring data for the U.S. population from the urine biomonitoring data for the 2009-10 NHANES cohort for 106 environmental chemicals. In this project, we updated the median human exposure data from the urine biomonitoring data for the 2009-10 NHANES cohort for 106 environmental chemicals. This study, we developed the median human exposure data from the urine biomonitoring data for the 2009-10 NHANES cohort for 106 environmental chemicals. In this project, we updated the median human exposure data from the urine biomonitoring data for the 2009-10 NHANES cohort for 106 environmental chemicals. This abstract does not necessarily reflect US EPA policy.
Per- and polyfluoroalkyl substances (PFAS) are of high public interest due to widespread production, environmental persistence, and adverse ecological and health impacts. EPA’s Comptox Chemicals Dashboard has published an extensive list of over 8000 curated PFAS structures. Whereas most studies to-date have focused on the health effects of a small number of PFAS compounds, such as PFOA and PFOS, relatively little is known about the health effects of the vast majority of PFAS and their byproducts. Methods for profiling the PFAS chemical structure space are needed to support modeling and structure-based categorization efforts. However, naming conventions and publicly available molecular fingerprinting methods are ill-suited to capturing the wide range of potentially relevant PFAS structural patterns. Expert-defined PFAS chemical category terms are limited to simpler categories (e.g., perfluorocarboxylic acids) and often lack clear structure definition. With the publicly available CSRL (Chemical Subgraphs and Reactions Markup Language), we developed a set of 130 TSPFAS fingerprints, including an expanded set of ToxPrint functional groups, augmented by over 80 new fingerprints capturing higher level categories, as well as unique aspects of PFAS structures, including perfluoro chains, polyfluoro substructures, fluorinated rings, and various perfluoro branching patterns. These CSRL PFAS categories and features can be processed with the public Chemotyper, provide comprehensive coverage of available PFAS lists, and are being used to profile and describe PFAS chemical lists currently undergoing testing within EPA. Abstract does not reflect US EPA policy.

**2566 Computational Associations between Obesity and Lead via Gut Microbiome Alterations and Impaired Expressions of PPARgamma and MC4R**


Obesogens are environmental chemicals that promote obesity, often by way of impacting adipocyte production or alternating metabolic setpoints and the hormonal regulation of satiety. Previous research and studies have demonstrated that there is an association between increased blood lead levels and obesity, but there is a lack of research detailing potential pathways. This study proposes and outlines additional computational associations between lead exposure and obesity and identifies the possible molecular mechanisms by which lead-gene interactions promote the onset of obesity or the development of precursory symptoms. More specifically, it examines potential links between lead exposure, epigenetic changes, and obesity by investigating lead exposure and alterations to appetite, satiety, energy utilization, and the gut microbiota. Online databases, published literature, and biomonitoring reports were utilized to establish the proposed chemical-gene-disease associations. Analysis of released chemical reports from United States counties with high rates of obesity demonstrated that lead was a common environmental contaminant in the majority of these areas. Preliminary chemical-gene-disease networks further showed that increased or decreased gene expression brought on by prominent chemical-gene interactions exacerbated symptoms linked to the onset of obesity. Of major interest was the effect of lead on Vitamin E absorption, PPARgamma, and MC4R expression and the resulting impact on gut microbiota regulation and satiety, respectively. Based on comparable literature and networks, two potential mechanisms by which lead exposure causes obesity were proposed. In the first pathway, lead exposure inhibited Vitamin E absorption in the gut microbiome, leading to a reduction in the microbial diversity of the digestive system whereby persistent oxidative stress ensues. In the second example, the increased proliferation of adipocytes as a result of PPARgamma overexpression is presented as well as the potential of lead to dysregulate feeding behavior through a reduction in MC4R.

**2567 New Public CSRML-Based Structure-Fingerprint Method for Profiling and Categorizing PFAS Structures for Modeling and Read-Across**

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Perfluoroalkyl substances (PFAS) are of high public interest due to widespread production, environmental persistence, and adverse ecological and health impacts. EPA’s Comptox Chemicals Dashboard has published an extensive list of over 8000 curated PFAS structures. Whereas most studies to-date have focused on the health effects of a small number of PFAS compounds, such as PFOA and PFOS, relatively little is known about the health effects of the vast majority of PFAS and their byproducts. Methods for profiling the PFAS chemical structure space are needed to support modeling and structure-based categorization efforts. However, naming conventions and publicly available molecular fingerprinting methods are ill-suited to capturing the wide range of potentially relevant PFAS structural patterns. Expert-defined PFAS chemical category terms are limited to simpler categories (e.g., perfluorocarboxylic acids) and often lack clear structure definition. With the publicly available CSRL (Chemical Subgraphs and Reactions Markup Language), we developed a set of 130 TSPFAS fingerprints, including an expanded set of ToxPrint functional groups, augmented by over 80 new fingerprints capturing higher level categories, as well as unique aspects of PFAS structures, including perfluoro chains, polyfluoro substructures, fluorinated rings, and various perfluoro branching patterns. These CSRL PFAS categories and features can be processed with the public Chemotyper, provide comprehensive coverage of available PFAS lists, and are being used to profile and describe PFAS chemical lists currently undergoing testing within EPA. Abstract does not reflect US EPA policy.
methods. Many factors could be identified that can affect the MPs sampling also. To assess the reliability of the obtained information about physicochemical properties of MPs, we prepared MPs sample in the laboratory and using analytical techniques i.e. FTIR, IR, SEM, SEC, and mass spectrometry established the similarity of the results to previous reports. For example, we could prove that FTIR spectroscopy can indeed be used for analysis of MPs having a size greater than 20 µm. We conclude that different MPs, dependent on their shape and size, do require analysis by a specific method. Unfortunately, quantification of a MPs mixture was still challenging although development of an accurate method that can quantify MPs in an environmental sample is crucial. Finally, we believe that the evaluation of MPs in any sample must be performed by a specific method to minimize the variability due to methods of separation and detection.

2570 **Health Risk Assessment Challenges of Handling and Disposal of Electronic Waste (E-waste)**

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Recent technological advances are generating considerable volumes of electronic waste (E-waste) resulting in one of the fastest growing pollution problems around the world. E-waste could be broadly categorized as large and small household appliances, information and telecommunication gadgets including various electric and electronic consumer products. We identified current trends of its export to developing countries, performed literature review of pollutants, and conducted analysis of the impact of E-waste exposure on human health. This includes production and sources of E-waste, improper recycling practices in developing countries and their toxic impact on human health. E-waste and its recycling produce a mixture of chemical contaminants such as metals and metalloids, persistent organic pollutants, dioxins/dibenzoofurans, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, bisphenol, styrene etc. Workers and their families at recycling and disposal facilities are exposed to the mixture of these pollutants through air, water, soil, or via different routes such as inhalation, dermal, transplacental, lactational, etc. Risk evaluation and risk management of E-waste remains a great challenge due to the complex nature of chemical mixture exposure and its potential adverse health effects in susceptible populations, especially women and children. Current human health risk evaluation approaches of E-waste pollutants are fragmentary and there exists no systematic risk assessment framework. It is essential to reduce or mitigate the negative effects of E-waste in a circular economy. We propose a general and site-specific exposure source-to-health outcome pathway and a tiered decision-making approach as a basis for cumulative and aggregate risk assessments of E-waste. It is based on operating conditions with considerations on types and quantities of E-waste received, stored, and disposed; nature of pollutants and monitoring data, exposure scenarios and their known or potential health effects on the most vulnerable populations residing at or near the recycling facility and community. **Disclaimer:** Authors have no conflict of interest and views/opinions expressed are of their own and do not represent policy or endorsement of their affiliations.

2571 **Health Assessment of Sodium and Potassium Salts of Inorganic Phosphates**

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When assessing the human health effects of phosphorus (P), specifically the sodium and potassium salts of inorganic phosphates, there are two notable departures from the traditional risk assessment approach to consider: 1) background concentrations found in humans are not zero, and 2) background concentrations found in contaminated sites are not zero. Phosphorus, as commonly found in various phosphates, exhibits a "U-shaped" dose-response curve, where dose levels above deficiency thresholds, and dose levels below toxicity thresholds, overlap across some population subgroups. Thus, care must be taken in the interpretation of extraneous exposures above a standard American diet that may result from exposures at sites contaminated with phosphate at levels above background. Phosphorus is most commonly found in its pentavalent form in combination with oxygen, known as phosphate or orthophosphate anion (PO43-). Phosphorus is an essential constituent of all living organisms, and its chemical forms and concentrations are quite uniform across most plant and animal species. Data from acute human clinical exposures from bowel preparations show clear renal and gastrointestinal toxicity, but these studies are acute, and may not extrapolate to subchronic or chronic durations germane to human health assessments at contaminated sites. Human dietary studies also show renal, cardiovascular, and skeletal endpoints near the same exposure levels when measured as mg P/kg-day. However, the dosimetry in these studies was determined by dietary surveys, completed years after the exposures, and failed to determine the exact forms of Phosphate in the diet, thus the confidence in the exact dosimetry is low. There are, however, robust animal data from acute, subchronic, chronic and developmental studies in several species, with similar endpoints as in human studies. In particular, a subchronic dietary study by Ritkses-Hoitinga et al (2004), which showed nephrocalcinosis in rats and rabbits, under conditions where calcium/phosphate ratios were specified (calcium blocks P absorption) appears to be relevant. Several additional animal studies described similar endpoints at slightly higher doses that further support the relevance of this potential point of departure. Doses that produced renal effects in humans have been shown to exceed those that produced kidney effects in rats and rabbits. **The views represented in this abstract are those of the author and do not necessarily reflect those of the United States Environmental Protection Agency.**

2572 **Are Environmental Phenols and Parabens in Food Chain Risk Factors for Prostate Cancer?**

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Environmental Phenols (EPs) and Parabens (PB) are endocrine-disrupting chemicals (EDCs) exposing humans through various routes, including the food chain. However, there is a lack of risk assessment methods for EPs and PB mixture exposures associated with prostate cancer. This research aims to apply an innovative risk assessment approach to investigate the association of mixture exposures of EPs and PB, along with epidemiological covariates and biological target (prostate cancer). We used comprehensive NHANES (2005-2015) data to analyze EPs and PB levels in the urine samples (n=4592) of men ≥ 20 years of age. Men with self-reported prostate cancer in their lifetime and urinary concentrations with ≤ LOD level of detection (LOD) in their urine samples were used for association analysis along with the selected confounders (age, BMI, income, race/ethnicity, education level, alcohol use, smoking status, US birth, physical activity, eat frozen food, liver and kidney diseases, weight, LDL, HDL, total cholesterol, and Triglycerides). Arithmetic means One-way ANOVA, R², ORs: 95% CI was applied to measure the associations between EPs, PB, epidemiological variables, and prostate cancer. We found that the mean values of EPs and PB (except Butyl) level in the urine samples were significantly higher in prostate cancer cases (n=152) than non-cases (n=4440). Numerical variables (Total serum cholesterol, LDL, and triglycerides) were also significantly higher in the men who self-reported prostate cancer in their lifetime. PB (R² of 0.6; r of 0.9), Triclosan (R² of 0.9; r of 0.9), and Methylparaben (R² of 0.8; r of 0.8) showed a better fit for linear regression model and very high positive correlation with (wight, LDL cholesterol, and Triglycerides). After adjusting for numerical variables, EPs [OR of 1.6, 95% CI: 1.25-2.33] and PB [OR of 1.2, 95% CI: 1.06-1.28] showed a significantly higher association with a self-reported prostate cancer diagnosis. The present study is the first step of risk assessment (hazard identification), which demonstrates a significant association of EPs and PB with > LOD in urine samples (alone or in mixtures), combined with epidemiological and quantitative variables and self-reported prostate cancer cases in US men.

2573 **Species Differences in Phenobarbital-Mediated UGT Gene Induction in Rat and Human Liver 3D Microtissues**


Species differences in hepatic metabolism of thyroxine (T4) by uridine diphosphate glucuronosyltransferase (UGT) and susceptibility to thyroid hormone imbalance could underlie differences in thyroid carcinogenesis caused by hepatic enzyme inducers in rats and humans. To investigate this hypothesis we examined profiles of hepatic UGT induction by the prototypical CAR activator phenobarbital (PB) in rat and human liver 3D microtissues. The rationale for this approach was that 3D microtissues would generate data more relevant to humans. Rat and human liver 3D microtissues were exposed to PB over a range of concentrations (500 µM - 2000 µM) and times (24-96 hr). Microarray and proteomics analyses were performed on parallel samples to generate integrated differentially expressed gene (DEG) datasets. Bioinformatics analysis of DEG data, including CAR response element (CRE) sequence analysis of UGT promoters, was used to assess species differences in UGT induction relative to CAR-mediated transactivation potential. A higher proportion of human UGT promoters were found to contain consensus CREs compared to the rat homologs. UGTS 1a6, 2b17 and 2b37 were upregulated by PB in rat liver 3D microtissues, but not in human liver 3D microtissues. By contrast, human UGTs 1a8, 1a10 and 2b10 showed higher levels of induction (RNA and/or protein) compared to the rat homologs. There was general concordance between the presence of CREs and the induction of UGT RNA. As UGT1A and 2B
isofoms metabolise T4, these results suggest that differences in UGT induction could contribute to differential susceptibility to CAR-mediated thyroid carcinogenesis in rats and humans.

**2574 Investigating the Transfer Rate of Waterpipe Additives to Smoke as an Integral Part of Toxicological Risk Assessments**

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Waterpipe, also known as hookah, narghile or narghila, shisha or bubbly bubbly, is a tobacco-smoking device. Waterpipe tobacco is heated and consumed by a process of inhaling tobacco smoke, that bubbles through water before being inhaled. To date, limited studies have determined the transfer of waterpipe additives from tobacco to smoke. This study, however, was designed to investigate the filtration ability of the waterpipe’s bowl to determine consumer exposure to flavors in smoke, which is an essential requirement to complete the toxicological risk assessment of waterpipe flavors. Within this study, a standard smoking protocol was used to evaluate the transfer of > 50 additives from experimental and commercially available samples. The results provide evidence that transfer rate varies between 0-65% depending on the additive. Physicochemical information (including water solubility, partition coefficient, molecular weight, boiling point and vapor pressure) is presented alongside additive transfer rates which were analyzed as a means to aid in the understanding of any correlation that may be later used to predict transfer, also considering results from Eyrthropel et al. (2020). These findings underscore the complexity of flavor transfer and highlight the necessity of exposure assessment for meaningful waterpipe flavor risk assessments.

**2575 Zinc Deficiency Exacerbated Bisphenol A Toxicity in Rat Testis**


Bisphenol A (BPA), an endocrine disruptor extensively used in the manufacturing of plastics worldwide. BPA is known to cause reproductive toxicity in humans as well as in experimental animals. These impacts on male infertility risk in individuals under reproductive age. Zinc (Zn), an important trace element and involved in the development of gonad, maintaining the spermatogenesis processes, integrity and maturation of sperms, nucleic acid metabolism and cellular antioxidant activities. Due to Zn deficiency, the reproductive health of every third person in the world during the reproductive age is at risk. Present study was designed to explore the influence of two important determining factors, such as BPA exposure and zinc deficiency on germ cell development and male reproduction. Sprague Dawley rats (aged 4 weeks) were randomly divided into four groups; such as control (normal pellet diet and drinking water), BPA (100 mg/kg/day), Zn deficient diet (ZDD) (fed with ZDD) and BPA + ZDD for 8 weeks. Biochemical and oxidative stress levels were quantified. Sperm motility, count and sperm head morphology were evaluated. Histopathological findings of testes, epididymis and prostate were examined. Testicular DNA damage was evaluated by comet and halo assay, sperm and testicular apoptosis levels were quantified by TUNEL assay. Serum protein electrophoretic pattern, testicular 8-OHdG expressions by immuno-histochemistry, testicular protein expressions like Nrf-2, catalase, PCNA and decreased in dietary zinc deficient condition as evident by the decrease in body and organ weight, serum testosterone and increase of testicular MDA, Zn levels. Decrease in the sperm numbers, motility and increase in abnormal sperm heads were observed. Further, histopathological alterations in testes, epididymis and prostate, increase of sperm and testicular apoptosis, testicular DNA damage as well as perturbations in Nrf-2, catalase, 8-OHdG, PCNA and Keap 1 protein expressions were observed. The findings indicated that dietary Zn deficient condition could be one of the crucial physiological conditions, for the exacerbation of BPA toxicity in rat testes and epididymis.

**2576 Noncancer Health Effects from Exposure to PCB Mixtures**


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Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants historically used in a wide variety of industrial applications and are currently released inadvertently as a byproduct of some manufacturing processes. Although intentional PCB production has been banned in the U.S. and most of the world, humans are continually exposed to complex mixtures of PCBs. Given concern for public health implications of PCB exposures, many human and animal studies have been conducted to assess the potential for PCB exposures to result in adverse health effects. This scoping review identifies and catalogs these studies to identify areas of robust research, uncertainty, data gaps, and research needs. We developed a literature search strategy and a Population, Exposures, Comparators, and Outcomes (PECO) statement to facilitate subsequent screening and tagging to organize literature into a systematic evidence map of PCB mixture effects on health. A comprehensive literature search of multiple databases yielded 59,135 unique records. We used natural language processing and machine learning methods to prioritize 15,793 studies for title and abstract screening, and 1,474 studies were identified as meeting the PECO criteria (883 human studies; 591 animal studies). Studies were organized by health effect category (i.e., cardiovascular, dermal, developmental, endocrine, gastrointestinal, hematopoietic, hepatobiliary, immune, metabolic, musculoskeletal, nervous system, ocular, reproductive, respiratory, and urinary system), exposure condition, and species. Relevant study details (e.g., PCB mixtures measured/administered, endpoints assessed, exposure duration) were extracted into literature inventories to assess database characteristics, trends, and topics that would benefit from further research. The evidence mapping illustrated significant diversity in exposure contexts and endpoints examined across the database. There were > 40 studies identified to each health effect category, with the last two decades identifying > 5 studies each for hepatobiliary, nervous system, and developmental effects. However, it is important to note that database size is only one of several factors to consider in hazard assessment. Other contributing factors include study quality and consistency and coherence of effects observed across the database. Some smaller databases contained well conducted, unique observations that could be informative for hazard identification. Future work will focus on the evaluation of studies for sensitivity and risk of bias and on the extraction of data for use in evidence synthesis and integration.

**2577 Influence of Transcriptionic Descriptors on the Generalized Read-Across (GenRA) Performance**

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Read-across is a data gap filling technique utilized to predict the toxicity of a target chemical using toxicity data from similar analogues. Recent efforts such as Generalized Read-Across (GenRA) (Shah et al., 2016) facilitate automated read-across predictions for untested chemicals. GenRA aims to make predictions of toxicity outcomes based on “neighboring” chemicals characterized by chemical and/or bioactivity fingerprints. Here we investigated the impact of biological similarities (based on targeted transcriptomic data) on neighborhood formation and read-across performance in predicting hazard classifications (based on repeat-dose testing outcomes from US EPA ToxRefDB v2.0) using recently developed python package, genra-py. We treated HepaRG™ cells with 8 concentrations of 1,060 chemicals and measured the expression of 93 transcripts, which measure nuclear receptor activation, xenobiotic metabolism, cellular stress, cell cycle progression, and apoptosis. Transcriptomic similarity between chemicals was calculated using binary hit-calls from concentration-response data for each gene. We evaluated GenRA performance in predicting ToxRefDB v2.0 hazard classifications using area under the ROC curve (AUC) for the baseline approach (chemical fingerprints) versus transcriptionic fingerprints and a combination of both (hybrid). Overall, an increase in read-across performance was noted for various toxicity endpoints when using either transcriptomic or hybrid fingerprints over baseline. For example, for all liver endpoints, there was a 10% improvement in performance utilizing transcriptomic fingerprints and a 16% improvement with hybrid descriptors. We also see improved predictive performance using a combination of various chemical fingerprints (Morgan, Torsion Topological, and ToxPrints). Thus, integration of diverse descriptors, either bioactivity combined with chemical information or combinations of various chemical fingerprints, offer significant benefit in predicting in vivo toxicity outcomes. This abstract does not reflect US EPA policy.
Medicinal enzymes are biopharmaceuticals administered for treatment of a variety of medical conditions. Their immunogenicity and sensitization potential have been investigated in patients via parenteral dosing, but the risks of exposure to occupational populations remains largely unknown, and regulatory occupational exposure limits (OELs) for medicinal enzymes are not available. We propose a framework for establishing medicinal enzyme OELs using a decision tree approach that considers clinical data, sensitization and immunological indicators, and additional innovator handling experience. There are three possible outcomes from this decision logic: 1) Pharmacology effect level as the point of departure (POD) for the OEL, 2) Pharmacology effect level as the POD for the OEL with a 10-fold adjustment factor, and 3) The sensitization default value of 60 ng/m³ based on the Ceiling Threshold Limit Value (TLV) for subtilisin established by the American Conference of Governmental Industrial Hygienists (ACGIH). First, one considers if there are relevant and sufficient clinical studies; enzymes without such studies default to an OEL of 60 ng/m³. If adequate clinical data are negative for sensitization, pharmacology effect level is used as the POD. If there are adequate and positive clinical data for sensitization, the 60 ng/m³ default OEL for protein sensitizers is assigned. If clinical data are equivocal for sensitization, hazard indicators (e.g., exposure regimes, sensitive populations, enzyme source, and enzyme characteristics) are evaluated. Enzyme characteristics consider structural and functional epitopes based on predictive sensitization data. Enzymes without hazard indicators have pharmacology effect level as the POD with an adjustment factor of 10. Enzymes with hazard indicators default to an OEL of 60 ng/m³, unless innovator data or use experience indicates there is no sensitization hazard at higher concentrations. If the innovator data and use experience indicate no sensitization hazard, then pharmacology effect level as the POD with an adjustment factor of 10 is used. This decision logic allows for incorporation of clinical, in vivo, in vitro, and in silico data regarding enzyme sensitization potential and immunogenicity, and re-assessment as more data become available.

2579 Derivation of Subacute Guidance Values of Inorganic Metal Contaminants Controlled by the Drinking Water Quality Standards in Japan


In Japan, 51 items of contaminants of drinking water are controlled by the legally binding Drinking Water Quality Standards (DWQS). Out of 51 items, 6 inorganic metals, namely cadmium, chromium (VI), mercury, selenium, lead, and arsenic are listed in DWQS. Sources of water supply can be influenced by local weather or chemical accidents, and the concentration of contaminants may transiently exceed the level of the DWQS. In this study, we derived the subacute guidance values (SGVs), i.e. concentrations of drinking-water contaminants that will not affect human health for subacute exposure duration, for these 6 inorganic metals. To derive subacute reference doses (sARfDs), a total uncertainty factor of 100 was applied to the NOAEL or BMDL of each subacute study. Subsequently, SGVs were calculated by using the sARfDs with an allocation factor of 100%. The SGV of mercury was calculated as >10 times higher than the DWQS value because that of mercury was developed based on the limit of detection but not human health effects. The SGVs of cadmium and selenium whose DWQS values were derived by human epidemiological studies were calculated as 3-4 times higher than the DWQS values. On the other hand, the SGVs for arsenic, lead and chromium (VI) were no alternative but to be evaluated as same as the DWQS values. When a level of arsenic, lead or chromium (VI) exceeds DWQS value, immediate management action, such as suspension of water supply will be required. The SGVs we proposed in this study is considered useful for risk management of the metal contaminants in drinking-water. This study was supported by a Health and Labour Sciences Research Grant (19LA1005) from the Ministry of Health, Labour and Welfare, Japan.
2583  Surfactants Category: An Integrated Approach to Testing and Assessment (IATA) Involving New Approach Methods (NAMs) for Assessing Inhalation Risks under the Toxic Substances Control Act (TSCA)


Surfactants are chemicals used in industrial operations, occupational settings, and consumer products that may result in exposure and toxicity in humans. TSCA requires submission to the U.S. Environmental Protection Agency (EPA) of a premanufacture notice (PMN) for new chemicals prior to commercialization. EPA must review the PMN to determine whether the new chemical substance presents a reasonable basis for injurious effects to human health or the environment. While TSCA requires submission of extant toxicity data, it does not require generation of new data, and mandates that EPA reduce or replace vertebrate animal testing. EPA therefore relies on a variety of assessment approaches, including read-across in which existing toxicity data are used to assess the new chemical within chemical categories. A category is a group of chemicals with structurally similar physicochemical (PC) properties and whose toxicity follows relevant pathogenesis due to an analogous mode of action. We propose an IATA for evidence integration and assessment of inhalation toxicity from surfactants to establish a new TSCA chemical category. The IATA includes: PC properties to determine chemical inclusion/exclusion; NAMs or analogues for read across, deployment of dosimetry modeling, and use of NAMs to identify potential key events of toxicity pathways. The IATA provides a scientific assessment approach to integrate data needed to perform risk evaluation of surfactants while also meeting the TSCA requirements to reduce vertebrate testing. These views are those of the authors and do not reflect views or policies of the EPA or other respective organizations.

2584  Development of Emergency Response Planning Guideline Values for Monoethanolamine

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Accidents in industry, commerce and transportation may be accompanied by unanticipated and uncontrolled releases of hazardous chemicals which may persist for only a short time. Planning for these short-term exposures requires assessing risk for short exposure durations. The American Industrial Hygiene Association’s Emergency Response Planning (ERP) Committee develops ERP guideline (ERG) values for inhalation exposures of one-hour duration via a weight of evidence approach including review and deliberation on acute or short-duration dose response data. The predictive assessment approach focuses on effects indicative of three tiers of severity where ERPG-1 covers objectionable odors and effects that are mild and reversible; ERPG-2 covers effects that might be permanent or serious or would impair the ability to escape a contaminated environment; and ERPG-3 covers lethality and life-threatening effects. Monoethanolamine (MEA) is used as feedstock in the production of detergents, personal care products, emulsifiers, polishes, pharmaceuticals, corrosion inhibitors, and chemical intermediates. A few inhalation studies of MEA are available from rats, mice, guinea pigs, cats and dogs; some of these serve as the basis for the NIOSH Immediately Dangerous to Life and Health (IDLH), German occupational exposure limit (MAK), and the OSHA Permissible Exposure Limit (PEL) values. Regarding ERPG-3, guinea pigs demonstrate increased sensitivity to lethality, but for other species, generally vapors below saturation result in NOELs of approximately 500 mg/m3; 200 ppm was not acutely lethal. An inhalation study summarized by the European Chemicals Agency (ECHA) used 60 ppm as the highest dose, implying approximately 180 ppm as the LC50 were also survived 5 daily 6-hr nose-only 200 ppm exposures. Regarding ERPG-2, respiratory tract pathology was observed following 5, 6-hr exposures to 80 ppm but not 20 ppm, and irritation was observed after a 28-day exposure to 20 ppm but not 4 ppm. Regarding ERPG-1, dogs exhibited lethargy following 2-3 weeks of exposure to 12 ppm. POD values for (non)lethality at 180 ppm and lethargy at 12 ppm were reduced by a factor of 3 based on considerations of mode of action, and inter- and intraspecies differences to propose ERPG-3 and ERPG-1 values of 60 ppm and 4 ppm protective of lethality and mild reversible effects, respectively. An ERPG-2 value of 20 ppm was proposed as a one-third reduction of the ERPG-3 value and supported by respiratory tract findings in rats, consistent with ERPG policy.

2585  Selection of an Analogue Compound for Oral Risk Assessment of Benzo[e]pyrene (B[e]P)


The oral database for the target compound, B[e]P, is inadequate to support derivation of oral toxicity values based on compound-specific data. Therefore, a tiered approach relying on an assessment of structural, metabolic, and toxicological similarity between a target compound and potential analogues was utilized for oral risk assessment. The initial focus was on the identification of structurally similar chemicals with toxicity values from IRIS, PPTV, ATSDR, or Cal/EPA databases using ChemIDplus and the OECD Toolbox. Six structural analogues to B[e]P containing 2 or more benzene rings were identified that have oral noncancer toxicity values: benzo[a]pyrene (BaP), fluoranthene, pyrene, anthracene, fluorene, and acenaphthene. The four candidate analogues with higher molecular weights (BaP, fluoranthene, pyrene, anthracene) are expected to behave more similarly to B[e]P in the body than the lower molecular weight PAHs (fluorene and acenaphthene). Of these, B[a]P is the preferred structural analogue because its physical and chemical properties most closely resemble B[e]P. B[a]P is also the preferred metabolic analogue for B[e]P. While all PAH analogues will undergo oxidative metabolism, B[a]P generates reactive dihydrodiols and diol epoxides due to presence of a bay region. B[e]P, which also contains a bay region, also has the potential to generate these reactive metabolites, albeit in a limited capacity compared with B[a]P. Available oral toxicity values for candidate analogues range from 0.0003 mg/kg/day for B[a]P and acenaphthene based on neurodevelopmental effects to 0.3 mg/kg/day (subchronic) and 1 mg/kg/day (chronic) for anthracene based on lack of adverse effects. In the absence of repeated exposure oral toxicity data for B[e]P, there is no information with which to clearly identify a candidate analogue for oral toxicity comparisons. However, ToxCast indicates more commonality in bioactivity for B[a]P and B[e]P compared to the other PAH analogue compounds, which may suggest similarities in the mechanisms of toxicity for these compounds. Based on structural similarity, comparable physical-chemical properties, similar oxidative metabolism to reactive intermediates and ToxCast data, B[a]P is considered the most appropriate analogue for oral risk assessment of B[e]P.
Animal welfare initiatives are focused on refinement of rodent studies to build the weight of evidence such that the dog is not compulsory for agrochemical toxicology testing in the future. Toxicokinetic (TK) evaluations are integrated into short-term rodent and dog studies and can provide valuable data on kinetic nonlinearity and systemic exposure (AUC<sub>24h</sub>) to help determine the weight of evidence to justify removing the dog as a required species. Rat and dog TK data were generated for the florpyn máxima benzyl herbicide and sulfloxazin insecticide. The florpyn máxima benzyl TK data for both species indicate no observed toxicity up to the limit dose of 1000 mg/kg/day and sublinear kinetics at >300 mg/kg/day, which is the defined KMD (kinetically derived maximum tolerated dose). At 13-weeks of dietary exposure to 300 mg/kg/day, the AUC<sub>24h</sub> in male and female rats was 232 and 223 μg hr/g. The AUC<sub>24h</sub> for male and female dogs was 103 and 78 μg hr/g at dietary dose levels of 324 and 368 mg/kg bw/day. AUC<sub>24h</sub> data can support that systemic exposure to florpyn máxima benzyl via the primary metabolite, X,11438848, is lower in the rat compared to the rat at similar doses with no observed toxicity, indicating the rat could serve as the most sensitive species for risk assessment. For the case of sulfloxazin, the administered dose levels for the dog were lower (range of 1 to 6 mg/kg/day for 90-days, and 15 mg/kg/day for 28-days) compared to the rat (6-10 mg/kg/day for 90-days) but resulted in dose corrected AUC<sub>24h</sub> values in plasma that were 3-5 times higher in the dog (range of 20-32 μg hr<sup>1</sup> ml<sup>-1</sup> vs. 6-9 μg hr ml<sup>-1</sup> in the rat) due to slower elimination. The liver was identified as the target organ in rats, while there were no observed adverse effects in the dog, indicating major differences in species toxicodynamics, but that the rat could be considered more sensitive. For both agrochemicals, the rat study NOAELs were used for human health risk assessments. Implementing a decision tree approach comparing TK profiles and systemic toxicity from short-term rodent and dog studies can provide data to determine the most sensitive, and therefore, most relevant species for risk assessment and reduce unnecessary rodent studies from the toxicity assessments. This framework was applied to guide the establishment of species specific PBPK models as well as in vitro cell based assays to provide critical data on TK biomarkers, systemic exposure, and species sensitivity.

### Setting Occupational Exposure Limits for Antimicrobial Agents: A Case Study Based on a Quaternary Ammonium Compound-Disinfectant

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Antimicrobial agents are an essential tool in controlling the transmission of SARS-CoV-2, and guidelines on their use have been issued by various public health agencies. Through its Emerging Viral Pathogen Guidance for Antimicrobial Pesticides, the US EPA has approved numerous surface disinfectant products for use against SARS-CoV-2. Despite their widespread use and range of associated health hazards, the majority of active ingredients in antimicrobial products lack established occupational exposure limits (OELs) to assist occupational health professionals in characterizing risks from inhalation exposures to these chemicals. A framework was derived for establishing OELs for antimicrobial agents based on established approaches from various organizations and a weight-of-evidence approach to assist in the scientific interpretation of the available data. This framework involves 1) a screening-level toxicological assessment based on a review of the existing literature and recommendations, 2) identification of the critical adverse effect(s) and dose-response relationship(s), 3) identification of alternative health-based exposure limits (HBEI), 4) derivation of potential OELs based on points of departure and uncertainty, and modification of existing HBEI, and 5) selection of an appropriate OEL. This framework was applied to guide the establishment of an OEL for a disinfectant product containing quaternary ammonium compounds (quats). Despite quats-containing products constituting nearly half of all disinfectant products approved by the US EPA for use against SARS-CoV-2, no OELs for these compounds have been published. Three potential OELs for inhalation exposure were derived for the quats-containing product based on irritation toxicity data, developmental and reproductive toxicity (DART) data, and modification of an existing HBEI, which ranged from 0.1 to 0.7 mg/m<sup>3</sup>. The final selected OEL for the quats-containing product was 0.1 mg/m<sup>3</sup> as an 8-hour time-weighted average, derived from modification of an existing HBEI. This value represented the lowest resulting value of the three approaches, and thus, was considered protective of the critical effect of irritation and any potential for DART. This framework can assist risk assessors in establishing protective exposure limits for antimicrobial products.

### Analytical Method Development for Transfer Testing from Smokeless Nicotine Pouches and the Elution Profiles of Nicotine and Flavors under Different Conditions

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Nicotine pouches are pre-portioned sachets containing nicotine, but no tobacco leaf, stem, or tobacco sheet, and are thus sometimes described as a “tobacco-free snus”. In order to evaluate the safety of nicotine pouches, it is important to determine whether they deliver the desired levels of nicotine to the user during intended use. In this study, we developed a stable method to measure the transfer rates of nicotine and flavors under simulated use conditions. For the transfer test, a dipping method was performed for 60 minutes in artificial saliva using a BIO-DIS Reciprocating Cylinder Apparatus. The concentrations of nicotine and flavors were analyzed by LC-DAD/kg bw/day and mld respectively. The optimal dipping conditions were selected based on the relationship between the dipping and the nicotine transfer rates. Two commonly used artificial saliva compositions were compared in order to select the appropriate artificial saliva for transfer testing. The transfer rates of nicotine and flavors were examined in pouches with different water contents, pouch weights or nicotine strengths. For nicotine, the transfer profile was examined over a 14-day study period. The study design was successfully deployed in the development of multiple pipeline agrochemicals, and data from at least 3 compounds will be presented. The major advantage of this TRF study design is that a single study can provide information for dose selection for the subsequent toxicity study, thereby reducing the number of animals used.
Flavoring workers are often exposed to many different respiratory irritants and it is therefore difficult to identify specific dose response relationships for diacetyl and other α-diketones from the health surveys of these cohorts. Fortunately, an abundance of animal inhalation studies has evaluated respiratory responses following exposure to specific α-diketones. We used animal study results to develop a diacetone daily time weighted average (TWA) “no effect threshold,” for the development of serious bronchiolar and alveolar (i.e. deep lung) effects, including bronchiolitis obliterans (BO), in humans. For chronic diacetone exposures (up to a 45-year occupational lifetime), we relied on the recent 2018 NTP two-year rat inhalation study involving daily 6-hour exposures. Benchmark dose modeling for the alveolar fibrosis endpoint was used to determine the benchmark concentration associated with a 10% increase in risk (BMC10) based on the number of rats with a pathology score of 1 (minimal alveolar fibrosis) or higher, using a multistage dose-response model. The 95% lower confidence limit (BMCUL) was calculated as 34 ppm and then converted to an 8-hr TWA exposure of 25.5 ppm. A dosimetric extrapolation to account for species-specific differences between rats and humans was applied (Rhuman/rodent=0.16) to estimate the percentage of inhaled diacetone expected to penetrate to human alveoli, yielding a threshold concentration of 4.0 ppm. For subchronic diacetone exposures, we relied on an NTP 13-14 week rat inhalation study (2018) in which a clear no-effect level for bronchiolar and alveolar effects was observed at 50 ppm. Applying the same adjustment factors as above yields a threshold concentration for an occupational tenure of 9 years or less of 6 ppm. In summary, we determined subchronic and chronic effect threshold concentrations that do not pose a risk of significant deep lung effects (including BO) and that can serve as a point of departure for occupational and regulatory exposure assessment. Given the similar degree of biological re-activity of longer-chain α-diketone flavoring compounds (e.g., 2,3-pentanedione and 2,3-hexanedione), the effect thresholds for these other α-diketones would be the same if not much higher than that for diacetone.

A Systematic Screening Hazard Identification Process for Versatile Implementation in Bespoke Human Health Risk Assessment Paradigms

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The human health risk assessment paradigm is a process by which potential health risk from exposure to chemicals can be qualitatively or quantitatively assessed. Paradigms can be adapted for the product type, exposure routes, and numerous toxicological endpoints. The process is also fit for purpose depending on implementation in the product development process itself, such as candidate selection or regulatory approval. For many paradigms, the initial hazard screening step is a critical component that relies on published toxicological and exposure data to prioritize chemicals for evaluation and determine the next steps of the hazard identification process. However, the screening process can incorporate numerous data sources and a positive or negative outcome is often subjective and lacks consistency across reviewers. This process is designed to ensure hazard assessments are reproducible, documented, and reproducible. Cr(VI) can oxidize to form Cr(III) on the surface of a leather product, the density of leather, thickness of product layer on skin, and migration of skin contact with the skin using the Cr(VI) concentration in the consumer exposure levels were calculated by estimating the amount of Cr(VI)

Quantitative Risk Assessment of Skin Sensitization Induction from Hexavalent Chromium in Leather Consumer Products

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Repeated exposure to hexavalent chromium [Cr(VI)] can induce sensitization and elicit chromium allergic contact dermatitis (ACD). Trivalent chromium [Cr(III)] is used in the leather tanning process to impart resistance to mechanical action, stability and heat. Cr(III) can oxidize to form Cr(VI) on the surface of leather products. Recently, there have been concerns from the media and public over hexavalent chromium found in leather goods, such as shoes, gloves, watch straps, and other skin-contact leather items, at concentrations above the limit of 3 ppm (3 mg/kg) set by the European Union (EU). We performed a quantitative risk assessment to determine the ACD risk associated with consumer dermal contact with Cr(VI) in finished leather products. The consumer exposure levels were calculated by estimating the amount of Cr(VI) exposed to be in contact with the skin using the Cr(VI) concentration in the product, the density of leather, thickness of product layer on skin, and migration rate of Cr(VI) from leather. Dermal consumer exposure levels were calculated for leather footwear products that were reported to contain 1 to 62 mg/kg Cr(VI), or 0.00045 to 0.028 µg/cm². A sensitization assessment factor (SAF) that accounts for variability among subjects, products, and product use patterns was incorporated into the analysis. These levels were benchmarked against a no expected sensitization level (NESIL) of 1 mg/cm² for chromium to derive a margin of safety (MOS). Overall, the MOS values estimated for all leather products were above 1, demonstrating that these products were not likely to be associated with an increased risk of skin sensitization induction based on the exposures evaluated. Further, as the MOS is greater than 1 for the leather products, our results suggest that the current EU regulatory limit of 3 mg/kg is likely conservative and indicate that a much higher concentration of Cr(VI) is necessary to elicit a potential...
sensitization effect. Overall, this quantitative risk assessment approach can be utilized with other allergenic chemicals to understand the potential risk of sensitization from articles of clothing.

2595 Toxicological Ontologies: Moving from Concept into Practice

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The U.S. Environmental Protection Agency (EPA) has made significant strides in making the evidence supporting human health risk assessments open and available to researchers and to the public. A key challenge in managing the evidence base is standardizing how data are being captured and curated across multiple disciplines (often with unique terminology), while context is maintained in the integration and categorization of like information. Quantitative values are relatively unambiguous; however, what the numbers mean (measurement units, methods, and conclusions) are relayed in more variable verbal form. Synonymous terms (e.g., “red blood cell,” “erythrocyte,” and “RBC”), use of discipline-specific jargon, changing terminology (e.g., the historic use of Clara cells versus club cells), and non-standard descriptive language all can lead to confusion. More precise semantics and ontologies are being used to digitize and integrate these disparate verbal data. Many key resources are already in use (e.g., Medical Subject Heading (MeSH) terms, Unified Medical Language System (UMLS) codes, BioPortal, and the National Cancer Institute Thesaurus); however, they are designed to work independently of one another. Coordination of these resources to organize and categorize environmental health information in a more consistent manner is our current challenge. We have previously described a conceptual strategic approach to address this challenge. We now describe in detail how these concepts are actively being applied in ongoing human health risk assessments through two related elements: 1) Internal Standards - a controlled Environmental Health Vocabulary was developed to label adverse effects measurement data in the HAWC database while retaining the authors original descriptive terms (thus retaining linkage to the original work); and 2) Semantic Interoperability via Ontologies - annotation of terms in the controlled vocabulary to ontologies (initially, UMLS codes) using a combination of automated and curated processes has established relationships between terms via semantic indexing, thereby serving as a point of integration to other existing database management systems. Case studies applying these tools in ongoing assessments of human health risk will be presented to illustrate the conceptual strategic approach in action.

2596 Exposure and Risk Assessment of Metals used in Oxo-biodegradable Plastics in Consumer Products

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Oxo-biodegradable plastics, which contain metal-based prodegradant catalysts, are used in a variety of consumer products. However, little is known about the type and quantity of metals used. The purpose of this study was to measure the concentration of metals in 11 consumer products manufactured with oxo-biodegradable plastics and perform an exposure assessment to determine the health risk of exposure to metals through the use of these products. Wipe testing was performed to evaluate the potential for surface transfer of metals. Further, total metal concentrations were quantified with inductively coupled plasma mass spectrometry (ICP-MS). No metals evaluated were detected in wipe samples from any of the products. However, detectable levels of one or more metals were found in 9 out of 11 samples analyzed through ICP-MS, of which 5 samples were food contact products (grocery bags, floss packs, coffee capsules, straws, and breast milk bags). These 5 samples contained manganese, cobalt, iron, copper, chromium, nickel, and titanium, of which titanium was found at the highest concentration, followed by copper. The overall total detectable metal content in the 5 food contact products ranged from 35 mg/kg to 5473 mg/kg (mean: 2270 mg/kg). For each food contact product, a screening-level Estimated Daily Intake (EDI) was determined using the conservative assumption that 100% of the metals detected in each product would be bioavailable and based on conservative estimates of product usage for various age groups where applicable (e.g., infants, toddlers, children, adults). The EDIs for metals detected were below current industry Derived No Effect Levels (DNELs) for all products except breast milk bags. However, ICP-MS results indicate total metal content using an intense heat and acid digestion method, which is not representative of a realistic exposure scenario for breast milk bags under normal and recommended use. Our findings suggest that metal additives in oxo-biodegradable plastics likely do not pose a risk to consumers during typical use. However, further research including a higher-tier dose-response evaluation and assessment of the potential for metals to migrate out of the product and leach into food under normal use is needed to evaluate whether metal additives in oxo-biodegradable plastics pose a risk to consumers.

2597 Impact of Updated BMD Modeling Methods on Perchlorate and Chlororate Assessments of Human Health Hazard

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Several exposure limits for perchlorate have been developed based on an early key event, inhibition of radioactive iodide uptake (RAIU) by the thyroid. These assessments have used a variety of definitions of the point of departure. The current assessment revisited the modeling for inhibition of RAIU using state of the science methods. Bayesian hierarchical modeling was used to account for the repeated measures on the same individuals in the key dataset, and the underlying Beta distribution used for the modeling correctly reflected the bounding of RAIU between 0 and 1. We defined the BMR as a point value of 8% RAIU (rather than a change in RAIU), based on descriptions in the medical literature that RAIU below this value is considered abnormal. Because a definition of the BMR based on the mean response would correspond to about 50% of the population with a response below the BMR at the benchmark dose, we used a hybrid definition of the BMR. That is, the BMD was defined as the dose at which it was estimated that there would be a 10% exceedance of the population having RAIU of 8% or lower. Sensitivity modeling was also conducted for older subjects, who have lower baseline RAIU. The resulting point of departure based on the BMDL for the primary analysis was 0.03 mg/kg/day.

2598 Use of the Mucilair Airway Assay, a New Approach Methodology, for Evaluating the Safety and Inhalation Risk of Agrochemicals

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The US EPA has issued a directive to reduce mammalian studies by 2025. The application of new approach methodologies (NAMs) is being implemented to provide information on chemical hazard and risk assessment in support of this goal. To that end, in vitro methods such as Muclair™ are used to detect airway damage and acute irritation potential in agrochemicals. In this study, five agrochemicals including Adepidyn™ and four investigational chemicals (Chemical #1-4) were evaluated using Muclair™ to determine the need for future in vivo inhalation studies. Evaluation of irritation potential and tissue damage was determined via the following endpoints: transepithelial electrical resistance (TEER), lactate dehydrogenase (LDH) release, resazurin metabolism, and histology. Each agrochemical was applied using the Muclair™ to four tissues for 24 h at six concentrations (Adepidyn™: 0.005-0.5 mg/L; Chemical #1: 0.1-1000 mg/L; Chemicals #2-4: 31-317 mg/L). Vehicle control, positive control (sodium dodecyl sulfate, SDS, 4 mM) and untreated air liquid interface (ALI) control groups were tested in parallel. The control treatments (vehicle and SDS) performed as expected at each endpoint. Exposure to Adepidyn™ or chemicals #1-4 did not cause a change in TEER or LDH release. There were no changes in resazurin metabolism. Increased numbers of necrotic epithelial cells were observed in samples administered ≥126.27 mg/L of Chemical #3. At the highest concentration (317.17 mg/L) of Chemical #3, a minor increase in epithelial thinning was observed. For all compounds, the irritation potential panel and histological evaluation showed that there was no evidence of toxicity at any concentration tested. In conclusion, this study has demonstrated the utility of the Muclair™ assay as a modern in vitro technique for the pointed assessment of airway damage and acute irritation potential. The data derived from such assays has improved the design of further in vivo testing needed to identify respiratory hazards in the safety assessment of future agrochemicals.
The GreenScreen® for Safer Chemicals is a hazard assessment framework that assesses chemicals against 18 human health, environmental, and physical hazard endpoints based on hazard combinations, assigns to a benchmark (BM) score of 1-4, with 1 being most hazardous. ChemFORWARD is a recently launched on-line hazard repository that is based on the Globally Harmonized System of Classification and Labeling of Chemicals (“GHS”) and Cradle to Cradle Certified® (C2CC) Material Health Methodology, and considers routes of exposure and LD50 endpoints. ChemFORWARD assigns a band of A-C and F, with F being equivalent to a GreenScreen® BM-1 (“Avoid - Chemicals of High Concern”) and A being equivalent to a BM-4 (“Prefer - Safer Chemical”). GreenScreen® hazard assessments for 61 plasticizers in ToxServices’ online ToxFMD® Screened Chemistry Library were compared to identify key differences between the two methodologies. Eight of the plasticizers were also evaluated following the GHS/C2CC methodology in ChemFORWARD. Of the 61 plasticizers, 18% are GreenScreen® BM-1, 49% are GreenScreen® BM-2 (“Use but Search for Safer Substitutes”), 15% are GreenScreen® BM-3 (“Use but Still Opportunity for Improvement”), and 10% are GreenScreen® BM-3-DG, due to a data gap (DG) that prevents a GreenScreen® BM-4 score. Of the 30 BM-2s, 85% are BM-2e (defined as posing moderate hazards for carcinogenicity, genotoxicity, reproductive toxicity, developmental toxicity, and/or endocrine activity). However, only 8 of the BM-2s are assigned with high confidence. Of 8 plasticizers also assessed under ChemFORWARD, 5 are BM-2e plasticizers, and the ChemFORWARD C2CC and GHS ratings align with the sub-benchmark score. The BM-2e with moderate carcinogenicity and/or reproductive/developmental toxicity is rated x/c-CMR(2) because of GHS Category 2 classifications for at least one of those endpoints. One BM-2e with very high aquatic toxicity is rated C2CC x/c because of the red risk flag for environmental toxicity endpoints. One BM-3 and two BM-3DG plasticizers obtained B and A ratings, respectively, under ChemFORWARD. Unlike GreenScreen®, ChemFORWARD does not penalize hazard ratings for the endocrine data gap. Examining trends in GreenScreen® and ChemFORWARD-scoring paradigms for a set of plasticizers provides insight into the identification of safer alternatives and can inform the design of safer plasticizers.

Oral Risk Assessment of 3-Monochloropropane-1,2-diol (3-MCPD) Using Bayesian Model Averaged Benchmark Dose Modeling

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3-Monochloropropanediol (3-MCPD) is present in foods as a process contaminant in acid-hydrolyzed vegetable proteins and edible oils and fats. Oral exposure may also occur through ingestion of drinking water from water purification plants that use epichlorohydrin-linked cationic polymer resins. Therefore, an oral risk assessment was performed to establish allowable drinking water levels. Toxikokinetic data indicate that 3-MCPD is rapidly and efficiently absorbed through the gastrointestinal system, is widely distributed in the body and may accumulate in the cauda epididymis of the testes in male rats. Metabolism occurs by glutathione conjugation and formation of mercapturic acids as well as through progressive oxidation to β-chlorolactaldehyde. Therefore, an oral risk assessment was performed to establish allowable drinking water exposures. Three BM-2 es with moderate toxicity are rated C2CC c/b-CRE because of the yellow hazard rating for endocrine disruption. Three BM-2 es with moderate carcinogenicity, genotoxicity, reproductive toxicity, developmental toxicity, and/or endocrine activity. However, only 8 of the BM-2s are assigned with high confidence. Of 8 plasticizers also assessed under ChemFORWARD, 5 are BM-2e plasticizers, and the ChemFORWARD C2CC and GHS ratings align with the sub-benchmark score. The BM-2e with moderate carcinogenicity and/or reproductive/developmental toxicity is rated x/c-CMR(2) because of GHS Category 2 classifications for at least one of those endpoints. One BM-2e with very high aquatic toxicity is rated C2CC x/c because of the red risk flag for environmental toxicity endpoints. One BM-3 and two BM-3DG plasticizers obtained B and A ratings, respectively, under ChemFORWARD. Unlike GreenScreen®, ChemFORWARD does not penalize hazard ratings for the endocrine data gap. Examining trends in GreenScreen® and ChemFORWARD-scoring paradigms for a set of plasticizers provides insight into the identification of safer alternatives and can inform the design of safer plasticizers.

A Risk Assessment of Inorganic Mercury Renal Toxicity from Application of Skin Lightening Products from Multiple Countries

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The use of skin whitening products has become a common practice in many countries, particularly among women in Asian, African, and Caribbean countries. The international skin whitening market is growing quickly, and by some estimates will be worth more than $31 billion by 2024. Inorganic mercury is often added to skin whitening products as an active ingredient due to its ability to inhibit the production of melanin. To be effective, skin whitening cream ingredients must be absorbed into the skin. In this analysis, we calculated the suggested frequency of use ranged from once per week to three times per day. An abundance of case reports of renal effects among regular users of skin whitening products raises the question of potential mercury toxicity from these products, especially in countries where mercury content is unregulated in cosmetics. In this analysis, data was compiled regarding mercury concentrations in skin whitening products as reported in the peer-reviewed literature. Results included products from 33 countries and regions, and included a variety of products including creams, soaps, and scrubs. Products from Saudi Arabia, Thailand, and China contained some of the highest mercury concentrations, with maximum concentrations as high as 126,000 ppm reported. Among the products surveyed (n=321), 146 products (45.5%) contained mercury above 1 ppm, the FDA’s allowable concentration of mercury in cosmetics. The risk of renal toxicity resulting from the use of these products was determined by calculating the daily dose of mercury and comparing it to the reference dose (RfD) for inorganic mercury to calculate a margin of safety (MOS). Separate preliminary risk assessments were conducted for the various rinse-off products (e.g., soaps, scrubs) and leave-on products (e.g., cremes) based on data regarding the dermal absorption of inorganic mercury from the peer-reviewed literature. The MOS for all products that are typically rinsed off (e.g., scrubs) is less than 1.0, indicating limited to no potential for increased risk of renal toxicity. The MOS for more than half of the leave-on product types were below 1.0, indicating potential increased risk of renal toxicity. To our knowledge, this is the first risk assessment to incorporate mercury concentrations from such a wide range of skin whitening products, and the results elucidate the likelihood of renal toxicity following regular use of these products.

Quantifying Uncertainty in Interspecies and Intraspecies Extrapolation for Equitop doses Using a PBPK Model

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In traditional human health chemical risk assessments, one applies a series of uncertainty factors (UFs) to a point of departure (POD) external dose or concentration to obtain a reference dose (RfD) or concentration (RfC) that is assumed to be without appreciable lifetime risk for humans. All of the aforementioned quantities (PODs, UFs, RfDs, and RfCs) are scalars in the traditional paradigm, but the World Health Organization (WHO) International Programme on Chemical Safety (IPCS) has proposed a new risk assessment paradigm designed to determine a probabilistic RfD or RfC for any given chemical. In the IPCS approach, assessment factors (AFs) described by probability distributions rather than scalar UFs are applied to a distributional estimate of POD such as one might obtain through dose response modeling. Just as some of the traditional UFs account for interspecies (e.g., rat-to-human) and intraspecies (e.g., average-to-sensitive-human) differences in pharmacokinetics (PK) and pharmacodynamics (PD), the IPCS method incorporates corresponding AFs for interspecies and intraspecies differences. IPCS has proposed a set of AFs described by lognormal distribution that is sought to determine whether the lognormal assumption is appropriate for PK AFs by using PBPK models together with information about uncertainty and variability in various anatomical and physiological quantities to perform interspecies and intraspecies extrapolations. As an illustrative example, we constructed probability distributions for the fraction of renal clearance of inorganic mercury and the renal mercury concentrations following the urinary excretion of inorganic mercury. Using the mass balance equation and renal mass, we estimated the dose delivered to the kidney and calculated the renal concentrations. As some of the traditional UFs account for interspecies (e.g., rat-to-human) and intraspecies (e.g., average-to-sensitive-human) differences in pharmacokinetics (PK) and pharmacodynamics (PD), the IPCS method incorporates corresponding AFs for interspecies and intraspecies differences. IPCS has proposed a set of AFs described by lognormal distribution that is sought to determine whether the lognormal assumption is appropriate for PK AFs by using PBPK models together with information about uncertainty and variability in various anatomical and physiological quantities to perform interspecies and intraspecies extrapolations. As an illustrative example, we constructed probability distributions for the fraction of renal clearance of inorganic mercury and the renal mercury concentrations following the urinary excretion of inorganic mercury. Using the mass balance equation and renal mass, we estimated the dose delivered to the kidney and calculated the renal concentrations.

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2604 Derivation of an Oral Reference Dose for Drinking Water Treatment Polymers, Polyacrylic Acid, and AA-AMPS Co-polymer, Using a Class-Based Approach

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Polyacrylic acid (PA) and acrylic acid/2-acrylamido-2-methylpropane sulfonic acid copolymer (AA/AMPS) are polymers used as descaling agents and disinfectants in drinking water treatment systems. General population exposure to PA and AA/AMPS may occur through drinking water and may also result from their uses as secondary direct food additives, indirect food additives and ingredients in cosmetic products. An oral risk assessment was undertaken to establish allowable drinking water levels. Due to the lack of toxicology data for the AA/AMPS copolymer, the risk assessment utilized the available data on PA (MW 10000) as well as supporting data for AMPS monomer. The available human clinical and epidemiological studies of PA indicate low concern for dermal irritation, skin sensitization and respiratory irritation but were insufficient to support derivation of chronic reference dose; therefore, the risk assessment relied on the available animal studies. Repeated-dose animal data for PA included five subchronic and three chronic dietary studies in rats and dogs, as well as three developmental studies in rats in which the target organs for toxicity were the kidneys and gastrointestinal tract. Due to limited data reporting in the chronic studies, the oral RD was based on a NOAEL of 1000 mg/kg-day from a 13-week feeding study in rats where significantly increased kidney weights were observed in female rats at 3000 mg/kg/day in the absence of renal histopathology. Chronic NOAEL values were comparable suggesting a lack of temporal progression of the critical effect. Toxicity data for the AMPS monomer indicate low bioavailability and low toxicity, providing support for the reference dose derived for the target polymers as sufficiently protective. Applying a total uncertainty factor of 300x (3x interspecies, 10x intraspecies, 3x duration, 3x database) to the human equivalent NOAEL of 236 mg/kg-day, an oral RD of 0.8 mg/kg-day was determined for PA and AA/AMPS polymers. The resulting Total Allowable Concentration in drinking water was 5000 µg/L when applying a drinking water intake rate of 0.032 L/kg-day and a 26% relative source contribution factor.

2603 Evaluating the Impact of Endogenous Formaldehyde Production on Cancer Risk


Previously described biologically based dose-response (BBDR) models for the nasal carcinogenicity of inhaled formaldehyde (F) in F344 rats (Conolly et al., Toxicol. Sci. 73, 432-447, 2003) and humans (Conolly et al., Toxicol. Sci. 82, 279-296, 2004) did not consider the potential impact of endogenous production of F (endo) on the estimated tumor risk of inhaled F (exogenous: exog). These models incorporated data on DHA-protein crosslinks (DPX) resulting from exog F and associated these DPX with the probability of procarcinogenic mutation per cell division and thereby the risk of cancer. Recent studies (e.g., Lu et al., Chem. Res. Toxicol. 24, 159-161, 2011) identified deoxyguanosine (dG) adducts resulting from both endo and exog F. This update to the dosimetry model for endo and exog F in rats (Anderson et al. Toxicol. Sci. 118, 716-731, 2010; Campbell et al., Toxicol Sci 177, 325-333, 2020) incorporates regional nasal dosimetry predictions provided by computational fluid dynamics (CFD) modeling. While Lu et al. (ibid) focused on dG adduct formation, the current modeling assumed that endo F also forms DPX with the same rate constant as exog F. This new dosimetry model predicts DPX and dG adducts due to both endo and exog F in the nasal epithelium of F344 rats. Rates of adduct formation are proportional to the predicted intracellular concentration of acetaldheyde and are consistent with rat nasal epithelial dose-response data (0.7 to 15 ppm) for endo and exog dG adducts and for exog DPX. Background concentrations of endo DPX are predicted to be approximately 10-fold greater than concentrations of endo dG adducts. With inhaled F below about 1 ppm, the concentrations of endo DPX and dG adducts are predicted to exceed those of exog DPX and dG adducts, while above about 1 ppm, 6hr exposures are predicted in concentrations of endo DPX and dG adducts comparable to those of endo DPX and dG adducts. Thus, over the range of concentrations associated with nasal tumors in rats (6 to 15 ppm), exog DPX and dG adducts are predicted to predominate.

2605 Toward a Quantitative Adverse Outcome Pathway for Small Intestinal Tumors in Mice

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Three reference chemicals, the fungicides captan and folpet and the transition metal hexavalent chromium (Cr(VI)), served as the basis of our recently published cytotoxicity-mediated adverse outcome pathway (AOP) for small intestine tumors in mice (Bhat et al., CTR, 2020). Upon entering the duodenum, cytotoxicity to the villous epithelium is the molecular initiating event, as indicated by crypt elongation, villous atrophy/blunting, and other morphological changes. The first key event (K1E) in the crypt proliferation/hyperplasia, requires durations exceeding 6 or 12 months to create more opportunities for the second KE, spontaneous mutation/transformation that leads to SI tumors. While the AOP leveraged extensive target species- and site-specific molecular, cellular, and histological mode of action data across a wide range of exposure doses and durations, opportunities exist to advance this AOP toward a more quantitative AOP (qAOP), since qAOPs have been acknowledged by OECD as facilitating regulatory utility. Building qAOPs requires the underlying data to be incorporated into mathematical or computational models to quantitatively link exposure to one or more KE relationships that are predictive of the adverse outcome. For Cr(VI), rodent and human PBPK models exist that are capable of relating applied dose to internal doses in the SI. Herein we discuss the data and modeling needs to develop a qAOP for SI tumors in mice. The highly efficient homeostatic regulation of SI epithelial cell sloughing, regenerative proliferation, and repair involves replacing up to 100 cells per day on a normal basis. Models informing the qAOP should describe the relationship between tissue dose, morphological changes (e.g., crypt length), enteroocyte number, spontaneous mutation rate, and tumor risk in mice. Combined with duration of exposure, such relationship might be extrapolated across species to predict tumor risk in humans. Using Cr(VI) data to build a quantitative model for SI tumors could be the first step toward building a qAOP broadly applicable to other intestinal carcinogens.

2606 Impact of Post Hoc Analysis on Transcriptomic Point-of-Departure Estimates

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Transcriptomics and benchmark dose (BMD) analyses offer potential to derive point of departure (PoD) values from short term dose response studies for rapid assessment of chemical bioactivity. To reliably capture functional biological information from gene expression, an established best practice selects genes using both a statistical significance and a magnitude of change threshold. How robust ontology pathway BMD values are to different metrics is unclear. Leaving questions about how transcriptomic changes should be interpreted in hazard characterization. We used published transcriptomic datasets (NCBI GSE147072, Rat S1500+ TempO-Seq data) of 5 day in vivo rat exposures (liver and kidney) to assess the impact of several statistical significance methods to determine the impact of gene selection methods on pathway BMD/BMDL summary values. For the first two tests, which consider the a dose-response in aggregate, we used genes statistically significant (FDR<0.05) and with a fold change over 1.5-fold (FC>1.5). With DESeq2, we used the union of all genes significant at any dose across all 8 doses. All six compounds had many significant genes using DESeq2, or by a simple fold change filter of |FC|>2. Some samples failed to produce any significant genes using the Williams Trend test, and one sample (Bromodichloroacetic acid, liver) had no genes significant by ANOVA. With Di(2-ethylhexyl) phthalate, DESeq2 significant genes produced 26 enriched pathways (Fishers test p-value < 0.05 and at least 5 elements found). The ANOVA gene list resulted in 13 enriched pathways, all also part of the DESeq2 pathway list. Williams test genes produced 18 enriched pathways, including the 13 found in ANOVA pathways, and two additional found in the DESeq2 pathways. Three pathways were unique to the Williams test gene lists. Median pathway BMD values for the ANOVA, Williams (average of all pathway median values) and DESeq2 pathways (average of 20 pathways by lowest BMD) respectively, were 56.4, 80.1 and 40.0 mg/kg and BMDLs were 31.3, 41.0, 70.1 mg/kg. In contrast, when pathway enrichment was performed with genes with a fold change more than 2-fold (FC>2) the pathway summary (based on just 11 pathways enriched) produced a BMD of 186.1 and BMDL of 101.6 mg/kg. The established best practice of using both a statistical threshold and a magnitude of change threshold produces consistent ontology enrichment results as well as consistent transcriptomic pathway-based summary PoD (BMD/BMDL) values, independent of the specific significance test used.
As ethyl n-butyrate has been detected in the extractant water from drinking water system components, oral exposure to ethyl n-butyrate under occurs via ingestion of drinking water. Oral exposure to ethyl n-butyrate may also occur via ingestion of many foods due to its natural occurrence or use as a flavoring. Ethyl n-butyrate is a short-chain fatty acid ester that undergoes rapid hydrolysis in the GI tract to n-butyric acid and ethanol. As available repeat-dose toxicity data for ethyl n-butyrate are in chemical-specific risk assessment, analogue compounds were considered. Data on short-chain fatty acid ethyl esters were favored over data on other butyrate esters as ethyl esters share ethanol as a primary metabolite. Other butyrate esters may metabolize to methanol or butanol, which are more toxicologically potent than ethanol. The butyrate compounds were excluded from the assessment so that not induce endpoints inconsistent with those of ethanol. The use of ethyl ester data was also favored over data on n-butyric acid (butyrate). Butyrate is produced endogenously from dietary fiber in the gut. Based on many factors, about 500-600 millimoles of short-chain fatty acids are produced in the gut per day. Based on mass ratios of butyrate to ethanol and butyrate, this equates to about 55 mg/kg-day of butyrate for an 80 kg adult. These data support the assumption that butyrate is unlikely to drive toxicity at levels found following oral exposure to ethyl n-butyrate. Data on an analogue compound, ethyl acetate, were used to derive an oral reference dose (RfD) for ethyl n-butyrate. The RfD is based on a human-equivalent NOAEL of 200 mg/kg-day identified from a 90-day gavage study in Sprague-Dawley rats given ethyl acetate. Decreased body weight and altered organ weights were observed at the human-equivalent NOAEL of 800 mg/kg-day. Using a 30x uncertainty factor to account for intraspecies (10x) and interspecies (3x) variability, an RfD of 7 mg/kg/day was determined. This corresponds to a Total Allowable Exposure (TAE) of 40 mg/L in drinking water. While human epidemiological data reveal strong evidence of a relationship between exposure to ethanol and an increased risk of developing certain cancers, as well as fetal alcohol spectrum disorders should exposure occur during fetal development, the level of ethanol exposure resultant from the metabolism of ethyl n-butyrate at the identified RfD is well below the level of ethanol exposure required to observe these adverse effects.

Electronic nicotine delivery systems (ENDS) have grown in popularity since introduced to the US market in 2007. Early ENDS sought to mimic combustible cigarettes, utilizing simple device designs for ease of consumer use. Innovative, more complex device configurations have since been developed and range from small “puff” styles to large tank “mod” styles to devices with adjustable power settings and increased nicotine delivery. Although ENDS have potential for reduced harm compared to combustible cigarettes, ENDS consumers could have unique exposures, such as metals, from these devices. The US Food and Drug Administration (FDA) has identified cadmium, chromium, lead, and nickel as harmful or potentially harmful constituents (HPHC) found in tobacco products, including ENDS aerosol. Other metals have been measured in trace amounts in ENDS aerosol; however, the potential health effects from inhalation of those metals have not been fully evaluated. A systematic analysis of 22 published studies was performed to assess consumer exposures to 32 metals during the use of varying types of ENDS products. Potential consumer exposures were estimated using reported consumer factors (puffing topography). Estimated exposures and associated health risks to select metals (cadmium, chromium, lead, and nickel) for different ENDS types were compared to exposures reported for combustible cigarettes, as well as known health guidance values to characterize the relative and absolute risk for non-cancer and cancer health effects. Results showed that some metal exposures (i.e., nickel and chromium) were significantly higher than combustible cigarettes, largely due to these metals being non-detectable in combustible cigarette smoke. While other metals (i.e., cadmium and lead) were measured in ethyl n-butyrate at concentrations significantly greater than some ENDS products. Daily exposure levels exceeded guidance values established for cancer and non-cancer effects from certain device types (i.e., cig-a-like) but not others (i.e., heat-not-burn). Characterizing metals and other HPHC exposures and health risks from ENDS is important for assessing potential harm reduction of ENDS compared to cigarettes and appropriateness for the protection of public health.
P5 2611 Inhalation of Two Prop 65 Chemicals within Vehicles May Be Associated with Increased Cancer Risk

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Chemicals are listed on California’s Proposition 65 (Prop 65) for their potential to cause cancer or birth defects or other reproductive harm. The chemicals from this list are often detected within interior vehicle dust and air. Therefore, this study examined the potential risk associated with five Prop 65-listed chemicals detected within vehicle interiors: benzene, formaldehyde, di (2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), and tris(1,3-dichloro-2-propyl)phosphate (TDCIPP). Exposure estimates based on time spent within a vehicle were derived from a method of calculating estimated concentrations from the literature. Regulatory levels established by the California Office of Environmental Health Hazard Assessment (OEHHA) were then used to generate percent reference doses (%RFDs) for chemical-specific daily doses as well as determine the probability of risk (exceedance probability) as a function of %RFD for each chemical-specific daily dose. Based on our meta-analysis, benzene and formaldehyde were detected in vehicle interior air whereas DEHP, DBP and TDCIPP were detected in vehicle interior dust. Benzene and formaldehyde were the only two chemicals with an estimated %RFD > 100 across any of the commute times. For commute times of 20 mins or longer, the %RFD was >100 for maximum exposures based on the “maximum allowable daily level” for benzene, and for 95%-percentile exposures based on the “no significant risk level” for benzene and formaldehyde. Furthermore, the probability of exceeding 100% RFD was highest for cancer risks associated with benzene, followed by cancer risks associated with formaldehyde and the risk of reproductive and developmental toxicity associated with benzene. Lastly, within the entire state of California, the percent of commuters with a 10% probability of exceeding cancer risk associated with benzene or formaldehyde exposure was 78% and 63%, respectively. Overall, our study raises concerns about the potential risk associated with inhalation of benzene and formaldehyde for people who spend a significant amount of time in their vehicles, an issue that is especially pertinent to traffic-congested areas where people have longer commutes.

P3 2613 Derivation of a Proposed Asbestiform Tremolite NOAEL for Lung Cancer

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Asbestiform tremolite can be found in some chrysotile and vermiculite deposits and, consequently, may be present in some consumer products containing these materials. Inhalation of asbestiform tremolite has been identified as a possible risk factor for lung cancer; however, to our knowledge, there are no published evaluations of the exposure-response relationship between asbestiform tremolite and lung cancer in humans. The purpose of this study was to evaluate the potential exposure-response relationship between asbestiform tremolite and lung cancer using two distinct approaches. The first approach entailed a series of lung cancer risk at stratified levels of cumulative exposure among chrysotile and vermiculite cohorts working in mines and processing facilities (i.e., potential high-exposure settings). The second method utilized derived relative potency factors for asbestiform tremolite/chrysotile, and a published chrysotile no-observed-adverse-effect-level (NOAEL) for lung cancer of 89 to 168 f/cc-years. Results from the first approach yielded asbestiform tremolite NOAEL values for lung cancer of 0.5 to <2.6 f/cc-years and ≥1.05 f/cc-years from the vermiculite- and chrysotile-exposed cohorts, respectively. Using the second approach, Libby amphiboles (as a surrogate for asbestiform tremolite) were determined to be approximately 2.8- to 12.4-fold more potent than chrysotile for lung cancer development. Assuming a fraction of the total Libby amphiboles was asbestiform tremolite, a NOAEL range of 0.43 to 3.6 f/cc-years for asbestiform tremolite and lung cancer was calculated. The results using two separate, yet complementary, methods are consistent and, therefore, support a reasonable approximation of the asbestiform tremolite NOAEL for lung cancer in the range of 0.5 to <2.6 f/cc-years. Evidence of non-linear exposure-response relationships between airborne asbestos exposures and asbestos-related disease have been demonstrated. Thus, the derivation of asbestos NOAEL values provides useful benchmark comparisons for quantitative asbestos risk assessments.

P2 2612 A Web-Based Literature Identification Platform for the ECOTOXicology Knowledgebase, Powered by Deep Learning

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The ECOTOXicology Knowledgebase (ECOTOX) is a comprehensive, publicly available resource providing single chemical environmental toxicity data on aquatic life, terrestrial plants and wildlife. The database is updated quarterly, and to identify relevant references and extract pertinent data, the ECOTOX data curation pipeline employs a methodical process similar to initial stages of systematic review. This labor-intensive workflow requires curators to regularly evaluate tens of thousands of candidate references, the majority of which are then rejected as not relevant. After the careful review of hundreds of thousands of potentially relevant articles, the ECOTOX database currently contains data for 12,223 chemicals and 13,266 species of thousands of potentially relevant articles, the ECOTOX database currently contains data for 12,223 chemicals and 13,266 species, respectively. Overall, our study raises concerns about the potential risk associated with inhalation of benzene and formaldehyde for people who spend a significant amount of time in their vehicles, an issue that is especially pertinent to traffic-congested areas where people have longer commutes.

P4 2614 Revisiting the Testicular Toxicity of Cyanide: New Data and Weight of Evidence Review

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Following NTP testing of sodium cyanide (NaCN), EPA (IRIS 2010) identified male reproductive effects as the critical endpoint for oral cyanide toxicity. This led to a listing of cyanides (CNs) on Prop 65. Based on newly available data, the potential male reproductive toxicity of CNs was revisited. Systematic review identified no relevant human studies but 10 relevant animal investigations, 5 of which scored reliable without restriction (1) by ToxToxTool and were considered in this assessment. Two NTP 13-week drinking water (DW) studies conducted at NaCN doses of 0, 30, 100, and 300 ppm reported dose-related reduced cauda epididymal weights in rats and mice in conjunction with decreased water intake. Altered sperm parameters were seen in rats only, although the least confounded measures (sperm per gram tissue) were unaffected by exposure. Rat control epididymal weights were above the published concurrent NTP historical control data (HCD) range, but those of NaCN-treated animals were within HCD range, indicating no adverse effect of NaCN treatment. Treatment-related changes in mice were consistent with reduced water intake, as reported in the literature. A new 13-week NaCN DW study mimicked the NTP study using the same rat strain and doses with study enhancements, including individual housing, “paired water” controls, thyroid hormone determinations, and a post-treatment recovery period. There were smaller water consumption decrements at 300 ppm compared to the NTP study (11% vs 18%), and no treatment-related changes in male reproductive organ weights/histopathology or sperm. Thyroid hormones were unaffected. The remaining high-quality CN studies also reported no adverse effects on male reproductive organs, although not all of the parameters measured in the NTP and new studies were evaluated. In summary, the male reproductive system effects reported in the NTP study likely were not directly related to NaCN treatment and should not serve as the basis for CN human health assessments.
Electronic cigarettes (EC) are a popular alternative to traditional smoking. While the effects of nicotine and acrolein are very well characterized in the lung, the effects of the flavorants, commonly utilized in EC, are less understood. Additionally, health risks associated with EC are challenging to evaluate due to a large range of flavoring agents and combinations commonly in use. These flavorants are used widely in foods and are generally regarded as safe; however, studies looking at the effects of the flavorants including vanillin on lung metabolism and lung disease are limited. Vanillin, an aromatic aldehyde, is both an electrophile and a commonly used flavoring agent with reducto-active chemical properties and is expected to interact within biologic systems. In this study, we examined the effects of vanillin, used in EC on lung cell metabolism using high-resolution metabolomics (HRM). Human alveolar bronchial epithelial cells (BEAS-2B) were treated with varying concentrations of vanillin used in EC conditions and analyzed for HRM (n=6/condition). The results of the metabolome-wide association study (MWAS) show that increased concentrations of vanillin perturbed metabolites associated with energy, amino acid, antioxidant, and sphingolipid pathways in BEAS-2B cells. We also conducted HRM on human plasma exposed to 2nd hand smoke and EC from Metabolomics Workbench to validate cell data (n=73). These results confirmed the cell data and also show that vanillin elimination products were positively associated with cotinine, a marker of nicotine use and lung fibrosis status. Additionally, a MWAS of the glucuronidated product of menthol, menthol-glucuronide, was also found to be significantly associated with oxidative stress product formation and amino acid perturbation. Collectively, these results show that the reactive aldehyde vanillin as well as other flavoring agents are perturbing metabolic pathways previously linked to lung diseases, IPF, ARDS, and asthma, and suggests that these chemicals in EC can potentially cause or exacerbate lung pathogenesis. More extensive evaluation of vanillin and other flavoring agents are warranted to determine contributions to lung disease.

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Sponsor: C. Barton

To help understand the health risks associated with the JUUL Electronic Nicotine Delivery System (ENDS) products, four JUUL ENDS products and the reference 3R4F cigarette were tested for cytotoxicity in the in vitro neutral red uptake (NRU) assay. Each JUUL ENDS product was tested as an e-liquid and as aerosol condensates, collected on a Cambridge filter pad followed by an impinger filled with ethanol at 0°C, using intense and non-intense puffing regimens. Cigarette smoke was collected with a similar apparatus and tested as smoke condensate using the ISO 20778:2018 puffing regimen. The NRU assay was conducted in accordance with OECD TG129 using two cell lines: the murine fibroblast BALB/c 3T3 cell line and the human lung adenocarcinoma A549 cell line. No cytotoxicity was observed with the JUUL ENDS products, while the reference 3R4F cigarette was cytotoxic and at much lower concentration ranges compared to ENDS condensates (IC50s: 3T3: 3.00 ± 0.14 µg/mL nicotine, A549: 4.98 ± 1.57 µg/mL nicotine). The results demonstrated that the four tested JUUL ENDS products were significantly less cytotoxic than the combustible (3R4F) cigarette under the tested conditions.


Toxins in e-cigarette (e-cig) aerosols include heavy metals, nicotine, and volatile organic compounds which are thought to trigger cellular dysfunction in the conducting airways and the gas exchange regions of the lung by disrupting oxidative signaling. However, it is not known if the presence of flavorants like maltol affects the cytotoxicity of e-cig aerosols. Herein we used precision-cut lung slices (PCLS) to evaluate the effects of e-cig condensate on lung oxidative stress, cell viability, and functional activity, and the impact of chronic lung inflammation on these responses. PCLS are viable 3-dimensional tissue sections, comprised of all cell types and structural components present in the lung. For PCLS preparation, wild type (WT) and surfactant protein D

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Electronic nicotine delivery systems (ENDS) are growing in popularity as alternatives to tobacco smoke. E-liquids used for ENDS vapor generation typically contain food-safe flavoring agents, despite lack of evaluation for their impact on the airway. One such agent, maltol, is a naturally derived chemical with pro-oxidant and metal chelating properties. The aim of this study was to examine the impact of ENDS-based maltol exposure on metabolism of the bronchial epithelium. Human bronchial epithelial (BEAS-2B) cells were cultured and exposed to ENDS vapors. E-liquid (3:7 propylene glycol/glycerin) contained 100 μM nicotine ± 3.9 mM maltol (n = 6). In a chamber with continuous, unidirectional airflow, cells were exposed to 1 standardized puff of ENDS vapors each minute for 1 hour. Cell extracts were collected for high-resolution metabolomics and analyzed in triplicate using hydrophilic interaction chromatography and positive electrospray ionization. Mass spectral features were extracted by aplCMS and xMsAnalyzer. xmsPANDA was used to compare between treatments for features which differed significantly, using limma, and features with high predictive importance, using partial least squares analysis. A discovery false rate (FDR) was applied for multiple comparisons. Features selected by both comparisons were analyzed for pathway enrichment with mummichog v1. At FDR < 0.05 and VIP > 2, 520 metabolic features were altered with maltol exposure. Pathway enrichment analysis revealed enrichment of numerous amino acid pathways. These included aspartate and asparagine metabolism (p = 0.0025), alanine and aspartate metabolism (p = 0.0025) and glutamate metabolism (p = 0.0027). ENDS-based maltol exposure disrupts amino acid metabolism in the bronchial epithelium. Metabolic alterations resulting from ENDS maltol exposure may contribute to development of later pulmonary complications.
null (SPD−/−) mice, which exhibit chronic lung inflammation, were euthanized and lungs instilled with agarose. Lung slices (300 µM) were prepared and cultured for 24 hr in Dulbecco’s Modified Eagle’s Medium (DMEM). PCLS were then rinsed and incubated for 4 hr with DMEM containing e-cig condensate generated from 25-250 µL of e-cig fluid. Condensates were normalized to glycerin, a major component of the e-cig fluid vehicle (doses equivalent to 50-500 µM glycerin). E-cig condensate was prepared from vehicle, nicotine, or nicotine+menthol containing e-cig fluids. Toxicity was assessed by LDH, mitochondrial WST-1 metabolism, glutathione (GSH), ciliary beating frequency (CBF), and airway responsiveness to methacholine (MCH). In PCLS from both genotypes, a dose-dependent increase in LDH release and a decrease in WST-1 metabolism was observed after nicotine or nicotine+menthol exposure. Both condensates also caused a dose-related GSH depletion with no effect on the ratio of reduced to oxidized GSH. Depletion of GSH was more severe in PCLS from SPD−/− mice relative to PCLS from WT mice, indicating that inflammation exacerbates oxidative stress induced by e-cig condensates. At doses equivalent to 500 µM glycerin, both nicotine and nicotine+menthol caused a significant reduction in ciliary function as measured by CBF; additionally, airway responsiveness to MCh was suppressed by 30%. Taken together, these studies identify e-cig condensate nicotine and menthol components as major inducers of pulmonary toxicity and functional impairment and highlight the relevance of pre-existing inflammatory disease in the severity of their toxic effects. NIH P30ES005022.

**P3 2620 In Utero Exposures to E-cigarette Aerosol Imprint Molecular Signatures and Alter Lung Function in Neonatal Mice**

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Approximately 7% of pregnant women in the United States admit to electronic cigarette (e-cig) use during pregnancy, even though there is no evidence that e-cig use during pregnancy is ‘safe’. We previously showed at birth in mice that in utero exposures to e-cig aerosols altered lung structure and significantly down-regulated >80 genes of the WNT signaling pathway, which is critical for lung organogenesis. Little, however, is known about the effects of fetal exposures to e-cig aerosols on lung alveologenesis. Here, we tested the hypothesis that in utero exposure to e-cig aerosols impairs lung alveologenesis and pulmonary function in neonates. Pregnant BALB/c mice were exposed 2 hours/day from gestational days 1-20 to either filtered air or cinnamon-flavored e-cig aerosol (36 mg/mL of nicotine). Lung tissue was collected from offspring on post-natal day (PND) 5 and PND11, representing the initiation and mid-period of lung alveologenesis in mice. Lung function was measured at PND11. Exposure to e-cig aerosol in utero led to a significant decrease in body weights at birth, which was sustained through PND5, compared to air control mice. At PND5, in utero e-cig exposures dysregulated genes related to WNT signaling and epigenetic modifications, in both females (~120 genes, including Wnt2,6,9A,11 &16, Dmnt3a,3b, and Hdac2,8,9) and males (~40 genes, including Wnt6,11 and Hdac9). These alterations were accompanied by reduced collagen content at PND5 - a time point when secondary septation occurs in mice and collagen synthesis to support the formation of the alveoli is close to its peak. At PND11, in females, transcriptomic dysregulation associated with epigenetic alterations was sustained (22 genes, including Hdac8), while WNT signaling dysregulation was largely resolved (10 genes, including Wnt9A). Lung function testing revealed that in utero exposure to e-cig aerosol increased the Newtonian resistance of offspring at PND11, suggesting narrowing of the conducting airways. These data demonstrate that in utero exposure to cinnamon-flavored e-cig aerosol can alter lung alveologenesis and pulmonary function in neonatal mice. Moreover, the molecular signatures imprinted by the in utero e-cig exposure may reflect epigenetic programming affecting lung disease development later in life.

**P3 2621 Inflammatory Response Elicited by Propylene Glycol (PG/VG), Menthol, and Tobacco Flavored E-cigarette Aerosols in C57BL/6J and BALB/cJ Mice**


Flavored e-cigarettes (e-cig) have become popular among teenagers in recent years. Currently, in many states, most flavors are banned except tobacco and menthol flavors. We hypothesized that menthol and tobacco-flavored e-cigarettes differentially affect the immune-inflammatory response in a strain-dependent manner. C57BL/6J and BALB/cJ strains (male and female; n=4/group) were exposed to e-cig aerosols containing PG/VG (50:50), menthol (0 mg/ml and 24 mg/ml nicotine), and tobacco (0 mg/ml and 24 mg/ml nicotine) for two hours/day for 3 days using the Scireq inExpose exposure system (Puff profile: 2 puffs/min, 70 ml puff volume). Similarly, mice were also exposed to tobacco flavored e-cig aerosols for comparison with menthol-flavored e-cigs. Differential cell counts and inflammatory mediators were measured in bronchoalveolar lavage (BAL) fluid. Acute exposure to e-cig aerosol containing PG/VG, menthol without nicotine, and menthol with nicotine significantly increased the total cell counts in the BAL fluid in male and female of both mouse strains compared to tobacco flavor. PG/VG exposure caused the highest infiltration of total cell counts in the BAL fluid. Neutrophil counts were increased in both PG/VG and menthol (0 or 24 mg/ml) exposed male and female BALB/cJ mice. T-lymphocyte counts were significantly and differentially altered by menthol and tobacco flavors in both strains. E-cig aerosol containing menthol (0 and 24 mg/ml) differentially affect inflammatory cytokines, such as MCP-1, IFNγ, KC, TNFα, RANTES, and Eotaxin in both the mouse strains compared to air group control. Similarly, tobacco flavor (0 and 24 mg/ml) e-cigarette aerosol differentially affected IL-1β, TNF-α, IL-6, IL-10, IL-2, IL-13, IL-6, IL-4, IL-5, IL-1α,IL-10, IL-17A, GM-CSF, MIP-1α, IL-1, and MIP-1β were predominantly observed in C57BL/6J mice. E-cig exposure containing PG/VG alone and menthol without nicotine induced chemotaxis such as neutrophilia and inflammatory response may be predominantly Th1 driven. Tobacco and menthol flavors showed a strain-dependent inflammatory cytokine response. This study demonstrated the increased susceptibility to immune-toxicity by acute tobacco and menthol flavor exposures. This study was supported by NIH R01HL135613 and U54CA228110.
The health effects of long-term e-cigarette use are currently unknown. While most flavor ingredients used in e-cigarettes are “generally recognized as safe” (GRAS) for oral consumption, there are limited data to evaluate their inhalation toxicity. As toxicity testing of individual flavor ingredients or formulations is not always feasible or desirable, we used a structure-based grouping approach to select 38 flavor group representatives (FGR) on the basis of known and in silico predicted toxicological data. In this study, a mixture of these FGRs was tested in a 5-week dose range-finding inhalation study to select appropriate flavor dose levels that can be used in a future chronic inhalation study. A/J mice were whole-body exposed to aerosol, propylene glycol (PG), vegetable glycol (VG) and nicotine (N; 2% [w/w]), PG/VG/N aerosol with flavors at low, medium, and high concentrations (4.6-18.6% [w/w]), or mainstream smoke (MS) from the 3R4F reference cigarette for 6 h/day, for 5 days per week, for 5 weeks. The aerosol nicotine concentration was 15 μg/L. Respiratory tract irritation and inflammation were evaluated by histopathology and bronchoalveolar lavage fluid (BALF) analysis. Serum, nasal epithelia, larynx, and lung samples were collected for omics analyses. The mice exhibited no signs of severe acute toxicity post-exposure. In contrast to MS exposure, exposure to the flavor aerosols, even at the highest flavor concentration, did not cause notable lung inflammation, evidenced by the lack of immune cell infiltration in the BALF and histopathological evaluation. The moderate to severe adaptive changes in nasal and laryngeal epithelia seen in the MS group were absent or minimal in the flavor groups. Exposure to flavor aerosols had a modest effect on the levels of serum inflammatory mediators, genes and proteins linked to xenobiotic metabolism and oxidative stress response pathways in the nose, and lung inflammation and tissue remodeling networks. These responses were qualitatively similar to but substantially less extensive than those observed following MS exposure. Thus, the tested flavor concentrations did not result in severe subacute toxicity or respiratory tract irritation and can be considered suitable for use in a future long-term inhalation study to assess chronic toxicity and lung tumorigenesis in A/J mice.

2624 Chemical Adducts of Reactive Flavor Aldehydes Formed in E-cigarette Liquids Are Cytotoxic and Inhibit Mitochondrial Function in Respiratory Epithelial Cells

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Flavor aldehydes in e-cigarettes, including vanillin, ethyl vanillin (vanilla) and benzaldehyde (berry/fruit), rapidly undergo chemical reactions with the e-liquid solvents, propylene glycol and glycerol (PG/VG), to form chemical adducts named flavor aldehyde PG/VG acetals that can efficiently transfer to e-cigarette aerosol. The objective of this study was to compare the cytotoxic and metabolic toxic effects of acetals and their parent aldehydes in respiratory epithelial cells. Cell metabolic assays were carried out in bronchial (BEAS-2B) and alveolar (A549) epithelial cells assessing the effects of benzaldehyde, vanillin, ethyl vanillin and their corresponding PG acetals on key bioenergetic parameters of mitochondrial function. The potential cytotoxic effects of benzaldehyde and vanillin and their corresponding PG acetals were analyzed using the LIVE/DEAD cell assay in BEAS-2B cells and primary human nasal epithelial cells (NHpEC). Cytotoxic and metabolic effects of acetals in cell culture were compared using Click-IT Edu cell proliferation assay in BEAS-2B cells. Gene expression changes in respiratory epithelial cells from exposure to benzaldehyde and vanillin and their corresponding PG acetals were examined by RT-PCR. Compared with their parent aldehydes, PG acetals diminished key parameters of cellular energy metabolic functions, including basal respiration, ATP production and spare respiratory capacity. Benzaldehyde respiratory capacity impairment was more pronounced with PG acetals (10-10mM) increased cell mortality in BEAS-2B and NHpEC, compare with benzaldehyde. Vanillin PG acetal was more cytotoxic than vanillin at the highest concentration tested while both diminished cellular proliferation in a concentration dependent manner. These observations suggest that PG acetals differentially regulate transcriptional program leading to changes in several pro-inflammatory and antioxidant genes. Reaction products formed in e-liquids between flavor aldehydes and solvent chemicals have differential toxicological properties from their parent flavor aldehydes and may contribute to the health effects of e-cigarette aerosol and solvent chemicals have differential toxicological properties from their parent flavor aldehydes and may contribute to the health effects of e-cigarette aerosol and solvent chemicals have differential toxicological properties from their parent flavor aldehydes and may contribute to the health effects of e-cigarette aerosol.

2625 Zinc in E-cigarette (EC) Aerosols Induces Oxidative Stress, an Inflammatory Response, and Exosome Release in Human Bronchial Epithelial Cells


EC aerosols contain elevated concentrations of metals, including zinc, but the effect of these metals on the respiratory system are not well understood. Our hypothesis is that the concentrations of zinc in EC aerosols adversely affect cultured human bronchial epithelial cells (BEAS-2B) and inhibit their differentiation into squamous epithelium. Zinc (0.2 – 0.5 μg/mL) decreased mitochondrial activity in the NHpEC. Furthermore, zinc (10-10mM) decreased cellular proliferation in live cell imaging experiments. Fluorozin™, a fluorescent zinc probe, was used to track the movement of zinc inside cells. Within 24 hours of treatment, numerous vesicles containing zinc were present in the cytoplasm. To determine if uptake was via endocytosis, the cells were incubated for 24 hrs with Dextran-Alexa555™ and zinc, and then incubated in zinc probe for 30 minutes. The dextran and zinc vesicles were co-localized. Endocytosis was validated using Dyna64A™, an endocytosis inhibitor. Zinc was tracked through the endocytotic pathway using markers for late endosomes (Rab7), multivesicular bodies (TSG101), and exosomes (CD63). Exosomes were isolated from culture medium and analyzed using an ExoQuick kit and size and quantified using a NanoSight 300. Extracellular exosomes were lysed in RIPA buffer and released zinc was quantified using Fluoizin3™. 24 hrs of zinc treatment also increased IL6 in the culture medium, indicative of an inflammatory response. After 48 hrs, oxidized glutathione increased in the treated cell cultures. Similar uptake of dextran and zinc was observed when exposed to zinc at the air-liquid interface (ALI) using a cloud chamber. BEAS-2B cells
treated with zinc at concentrations present in EC aerosols rapidly internalized extracellular zinc and packaged it into exosomes, which were secreted, while at the same time releasing an inflammatory cytokine, and showing evidence of oxidative stress. These data suggest that chronic zinc exposure could damage the respiratory epithelium and lead to disease.

**2626 Dose and Effect of E-cigarette Vape in an Airway In Vitro Model**


E-cigarettes evaporate a liquid consisting of glycerol and propylene glycol, which often contains nicotine and flavourants. Many flavourants used in e-liquid have been tested for their toxicological effects for oral use, e.g. in food products, and have a GRAS status: Generally Regarded as Safe for oral use. However, inhaling such compounds could impose health risk because the exposure route is different and pyrolysis products may be formed in the e-cigarette. Identification of the most hazardous flavourants in e-liquids would allow informed decision making by users or regulation by governments. In vitro airway models were used to identify toxicological effects of one of the flavourants occurring in many e-liquid flavours: ethyl maltol. Epithelial cells form the first line of defense for inhaled compounds, many of which deposit in the bronchial tree. Human bronchial epithelial cells (Calu-3 cell line) were cultured at the air-liquid interface and exposed to vape from e-liquids with or without nicotine (10mg/ml) and with or without ethyl maltol (10mg/mL). Cells were exposed (2 puffs/min, 55ml/puff, 3 sec per puff) once (30 minutes) or twice (60 minutes) per day for 2 consecutive days using a Vitrocell smoking machine (VC1). Exposed concentrations, measured in the air just above the cells, was found to be approximately half of the nominal concentration (based on e-liquid usage). In addition, translocation was measured in the basolateral compartment during the post-exposure period. Other parameters, such as cell viability and epithelial membrane integrity, indicated no effect of the exposure. IL-8 production was affected in exposed cells, which was more pronounced after two exposures per day compared to a single exposure per day. Further characterization of the toxicity will include assessment of other cytokines. Our data show an effect of exposure to e-cigarette vape, but more testing is required to determine whether nicotine and ethyl maltol may have adverse effects on other parameters or after prolonged exposure. Also, we found translocation of nicotine and ethyl maltol across the cell barrier, which could be an indicator of absorption of these compounds.

**2627 Toxicity of Flavor Chemicals and Reaction Products in E-cigarette Fluids and Aerosols**


Electronic cigarettes (ECs) heat a mixture of solvents, nicotine, and flavor chemicals to form an inhalable aerosol. Heating causes chemical reactions resulting in the production of numerous reaction products. Our goal was to evaluate the toxicity of ethyl maltol and methylglycolyl (MGO), a commonly used flavor chemical and a reaction product of solvents, respectively. Both chemicals showed a high level of cytotoxicity in the MTT assay. Human bronchial epithelial cells (BEAS-2B) were exposed either in submerged culture or at the air-liquid interface (ALI) using the VitroCell™ cloud chamber system. In the ALI model, cells were exposed to ethyl maltol twice and labeled with MitoTracker™ Red to evaluate mitochondrial membrane potential. Exposure to 0.1 mg/ml of ethyl maltol elevated mitochondrial membrane potential above the untreated controls, while 10 mg/ml of ethyl maltol caused a dramatic loss of fluorescence. Using these two concentrations, BEAS-2B were exposed twice to ethyl maltol in the VitroCell™ and used for label-free proteomics. Pathways that were significantly changed by ethyl maltol treatments included inflammatory responses, cell cycle regulation, fatty acid metabolism, and survival and stress. Submerged treatments with MGO promoted caspase mediated apoptosis, with a significant induction of caspase-3 at very low concentrations (0.001mg/ml) of MGO. Both chemicals caused an inhibition of IL-6 and IL-8 secretion when exposed in submerged culture and in the VitroCell™ cloud chamber. These results show that acute exposure to ethyl maltol or MGO at concentrations found in EC aerosols impairs the health of bronchial epithelial cells, by interfering with mitochondrial function and inducing cell stress (ethyl maltol) or activating apoptosis (MGO). Chronic exposure to these chemicals will likely exacerbate these effects and may compromise human health.

**2628 Metal Analysis of E-liquid and Vapor from Stainless Steel and Nichrome Atomizers at Low and High Wattages**


Electronic cigarette (e-cig) use has increased in recent years, partly due to the perception that it is a safer alternative to tobacco cigarettes. E-cigs deliver nicotine, but also other potentially harmful compounds known to cause adverse health effects. Toxins are found in e-liquid, e-vapor, and on device components. Previous work determined that rats acutely exposed to e-cig vapor using a nickel chromium (NC) compared to a stainless steel (SS) atomizer at 45 watts (W) experienced significant acute e-cig or vaping product use-associated lung injury (EVALI) and inflammation in lungs and nasal airways. This study characterized and quantified toxic metals in e-liquid and e-cig vapor from atomizers using SS or NC heating coils powered at 45 or 70 W to determine if metals contributed to EVALI. Samples of e-liquid with a 50/50 propylene glycol/vegetable glycerin ratio containing tobacco flavoring without nicotine were collected at three timepoints: before and after being heated, and the vapor condensed. Samples were diluted to a 1:100 ratio using 1% nitric acid and analyzed for metals using inductively coupled plasma mass spectrometry. Chromium (Cr) concentrations in all sample types were 12 - 14 (relative standard deviation, >2%) μg/l (ppb). Going from 45 to 70 W increased the Zinc (Zn) concentration in vapor from 8 (1.4) to 88 (1.7) ppb for SS and 4 (1.3) to 13 (1.7) ppb for NC. Zn concentration in non-heated e-liquid was 1 -2 ppb (3.5) while heated e-liquid was 9 (0.9) ppb for SS and 18 (1.6) ppb for NC at 45 watts, and 4 (1.1) ppb for SS and 2 (1.3) ppb for NC at 70 watts. Although copper and nickel were not detected in vapor, low concentrations of 1-5 (1-7) ppb in both e-liquids with heated e-liquids slightly higher suggests metals leaching from the atomizer. Iron, cobalt, silver, and cadmium were not detected. Other compounds found in e-liquid and vapor that were not analyzed in this study may contribute to EVALI and may also contribute to the difference in symptoms seen between NC and SS at high wattage. E-cig users should be aware that potentially toxic metals are present in e-liquids, leach from atomizers and increase at high wattage. Overall, composition of the atomizer in an e-cig causes variation and increases in the metal concentration found in inhaled e-liquids, potentially influencing disease outcomes.

**2629 Miro1 Regulates Mitochondrial Dysfunction and Inflammation by Cigarette Smoke and Environmental Tobacco Exposure in Lung Epithelium**


Cigarette smoke (CS) is known to cause mitochondrial dysfunction which plays a cardinal role in the initiation and progression of several lung diseases including Chronic Obstructive Pulmonary Disease (COPD). Rho1 (Miro1), a calcium-binding, membrane anchored GTPase necessary for mitochondrial motility on microtubules which plays a key role in mitochondrial turnover by PINK1/Parkin mitochondrial quality control system. We have recently shown that CS decreases Miro1 abundance in lung epithelial cells. However, the role of mitochondrial Miro1 in CS-induced mitochondrial dysfunction and ensuing lung inflammation leading to the progression of COPD is not known. Hence, to determine the role of Miro1 in CS-induced lung inflammation, we exposed epithelial-cell specific conditional Miro1 knockout (Miro1CreKO) mice to environmental tobacco smoke (ETS) (<10 mg/m³) for 3 months and mainstream CS (~300 mg/m³) for 3 days duration. ETS exposure did not show any significant changes in the total cell count and neutrophil influx in bronchoalveolar lavage fluid (BALF) in both WT C57BL/6J and Miro1CreKO mice as compared to air controls. However, there was an increased levels of TNF-α, MCP-1 and MIP-1α in BALF from ETS (3 month)-exposed Miro1CreKO mice as compared to WT controls. Furthermore, ETS exposure showed a non-significant increase in compliance and decrease in elastance in Miro1 conditional KO mice. Acute 3 day mainstream CS exposure showed a significant increase in the total cell count and macrophage numbers in BALF in both WT and Miro1CreKO mice. However, there were no any changes in the neutrophil, CD4 and CD8 T-cell counts in BALF from CS-exposed WT or Miro1 KO mice. Immunoblotting results showed a significant decrease in the levels of Miro1-associated mitochondrial membrane proteins, OPAL1 and Parkin, in lungs from CS exposed Miro1 KO group in comparison to WT mice. Overall, our results show that epithelial Miro1 ablation leads to disrupted mitophagy by alteration in the levels of mitochondrial membrane proteins, pro-inflammatory cytokines and lung mechanical property. Further work is in progress to determine the role of Miro1 in the pathogenesis of COPD. Supported by the NIH R01 ES029177 and HL137738.
Virtual 2021 SOT Annual Meeting and ToxExpo

2630 Comparative Assessment of Exposure Effects of Smoke from Reference Cigarettes 3R4F and 1R6F In Vivo

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Within the scientific community, reference cigarettes from the University of Kentucky are widely used for comparing experimental results on the effects of cigarette smoke (CS). Now, a new Kentucky reference cigarette (1R6F) has been manufactured to replace the previous reference cigarette 3R4F. The 3R4F has, to date, been used in many comparative in vivo toxicological assessment studies on cigarettes and novel tobacco products. Therefore, it is essential to evaluate the effects of 1R6F in comparison with the historical 3R4F data. As our main objective was not to formally test for differences, but for equivalence —and given the high number of simultaneously investigated endpoints, we used statistical equivalence principles to explore the similarities between the two reference cigarettes. To that end, we leveraged the historical 3R4F data from previous 90-day rat inhalation studies to assess the range of variation of 3R4F exposure and sized the equivalence limits for each endpoint. All exposures were targeted to achieve 23 µg/L nicotine in the test atmosphere. The variability of the measured compounds in the test atmosphere was low for 3R4F among the studies. With the exception of formaldehyde and acrolein, the 1R6F test atmosphere contained marginally lower concentrations of the measured compounds (e.g., nicotine). The breathing patterns of the animals did not vary between 3R4F and 1R6F CS inhalation. The concentrations of nicotine metabolites measured in urine in the 1R6F group were within the range of variability of those in the 3R4F group, but at the lower end. Inflammatory mediators selected for their unique expression in bronchoalveolar lavage fluid showed variations with the new reference product, which were comparable with the variations with 3R4F across the studies. In summary, the differences observed between the two reference cigarettes are modest, and, in most of the cases, within the range of variability of 3R4F alone. Therefore, the 1R6F reference cigarette is a suitable replacement for the 3R4F reference cigarette in comparative tobacco product assessments.

2632 Assessment of Oxidative Stress Response to Heated Tobacco Product Vapor Compared with That of Mainstream Cigarette Smoke in Human Bronchial Epithelial Cells

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Heated tobacco products (HTPs) that do not burn or combust have gradually spread to the global market. As HTP vapor contains fewer harmful and potentially harmful constituents than does mainstream cigarette smoke (MCS), HTPs can potentially reduce the health risks associated with smoking. One of our HTPs, Direct heating Tobacco System Platform 2 Generation 0 version a (DT 2.0a), directly heats the tobacco and carries the aerosol with the flavors and nicotine derived from tobacco leaves. To compare the biological impact of the DT 2.0a vapor with that of MCS, we compared the oxidative stress response, one of major reactions from MCS exposure, in BEAS-2B cells, which are immortalized human bronchial epithelial cells. The aqueous extracts (AqEs) were prepared by bubbling mainstream aerosol from the DT 2.0a or MCS from Kentucky reference cigarette, the 1R6F. Cell-viability assay were conducted to determine the suitable concentration ranges for each AqE. We then analysed the oxidative stress responses (intracellular ROS production the intercellular oxidized form of glutathione against glutathione (GSH/GSSG) ratio, the antioxidant response element reporter activity) and the interleukin (IL)-8 secretion levels in the BEAS-2B cells exposed to each AqE. The cell-viability assay showed that IC50 of the 1R6F AqE was approximately 100 times lower than that of the DT 2.0a AqE. Furthermore, the oxidative stress response assays showed that the DT 2.0a AqE had less impact on the oxidative stress response than did the 1R6F AqE. In addition, the DT 2.0a AqE induced IL-8 secretion at higher concentrations of the cell extract induced by the 1R6F AqE in summary, although the DT 2.0a AqE induced oxidative stress responses at higher concentrations, the effective dose range of the DT 2.0a AqE was much higher than that of the 1R6F AqE. Thus, the results of this study indicated that the biological impacts of the DT 2.0a was lower than that of MCS.

2633 Thirdhand Smoke and Nicotine Cause Phenotypic Changes in the Mitochondria of Human Keratinocytes


Thirdhand smoke (THS) consists of residual tobacco smoke that settles and remains on indoor surfaces after smoking has ceased. We previously showed that THS extracts altered mitochondrial phenotype in mouse neural stem cells (mNSC). The normally punctate mitochondria of mNSC were transformed to a networked phenotype by THS treatments. The purpose of this study was to determine how THS extracts affected mitochondria in differentiated human keratinocytes, which normally have networked mitochondria. THS impregnated fabrics were created in controlled laboratory conditions, and aqueous extracts of these fabrics or of unexposed control fabrics were tested in vitro. Prior to exposure, cells were transfected with MitoTimer, a fluorescent reporter to visualize mitochondrial morphology. Cells were treated for 24 hrs with 50%, 75% and 100% THS extracts. We further assessed the role of nicotine in mitochondrial dynamics by treating cells with 10, 100, and 400 µg/mL of nicotine for 24, 48, and 72 hrs. Treatment with THS extracts for 24 hours increased the percentage of punctate mitochondria, while simultaneously decreasing the networked morphology. Punctate mitochondria significantly increased in cells treated with both 10 and 100 µg/mL of nicotine at 48 hrs and 24 hrs, respectively. After 72 hrs all concentrations of nicotine caused a significant decrease in the networked phenotype. In western blots, keratinocytes exposed to 400 µg/mL of nicotine for 72 hrs showed a significant decrease in mitochondrial fission, which normally have networked mitochondria. The aqueous extracts of these fabrics or of unexposed control fabrics were tested in vitro. Prior to exposure, cells were transfected with MitoTimer, a fluorescent reporter to visualize mitochondrial morphology. Cells were treated for 24 hrs with 50%, 75% and 100% THS extracts. We further assessed the role of nicotine in mitochondrial dynamics by treating cells with 10, 100, and 400 µg/mL of nicotine for 24, 48, and 72 hrs. Treatment with THS extracts for 24 hours increased the percentage of punctate mitochondria, while simultaneously decreasing the networked morphology. Punctate mitochondria significantly increased in cells treated with both 10 and 100 µg/mL of nicotine at 48 hrs and 24 hrs, respectively. After 72 hrs all concentrations of nicotine caused a significant decrease in the networked phenotype. In western blots, keratinocytes exposed to 400 µg/mL of nicotine for 72 hrs showed a significant decrease in mitochondrial fission, significant increase in the networked phenotype. In western blots, keratinocytes exposed to 400 µg/mL of nicotine for 72 hrs showed a significant decrease in mitochondrial fission, significant increase in the networked phenotype.
The heated tobacco product (HTP) and electronic cigarette (e-cigarette) have been available in the market and the use of these products are rapidly increasing among youth and adults. Because HTPs do not actually burn tobacco, it significantly reduces the levels of toxicants derived from combustion in the aerosol, and it is considered have a low impact on health compared with traditional cigarettes. E-cigarette liquid(e-liquid) is mainly composed of glycerol, propylene glycol, nicotine and various flavors. It is found that the chemical composition of aerosol produced by e-liquid heating is quite different from that of traditional cigarette smoke, and the type and release amount of harmful components are significantly lower than that of cigarette. The mouse lymphoma assay (MLA) is used internationally for short-term mutagenicity tests, which has been considered as the most sensitive in vitro mammalian cell gene mutation assay and therefore is especially useful in evaluating substances with unknown or multiple genotoxic mechanisms. 2D 3RAF reference cigarettes were smoked according to the ISO condition (35 ml puff volume, 2 second duration, 60-second puff interval, with the ventilation holes un-blocked). 4 HTPs from domestic and abroad were smoked according to the Health Canada Intense (HCI) condition (55 ml puff volume, 2 second duration, 60-second puff interval, with 100% blockage of filter ventilation holes). 8 puffs of each HTPs were smoked, and 4 HTPs were collected in a Cambridge filter. A total of 20 HTPs were smoked from each product. 3 commercial e-liquids with nicotine concentration respectively for 6, 12 and 18 mg/ml were smoked with the same e-cigarette device for aerosol production, according to the HCI condition. After smoking, the cigarette smoke condensate (CSC), the smoke condensate (SC) of the aerosol for THPs and the e-cigarette aerosol extract (ECE) of cambridge filter was extracted with DMSO at 10 mg/ml, 30 mg/ml and 10 mg/ml, respectively. The results showed that the CSC, SC and ECEs showed cytotoxicity to the LS178Y cell. TFF resistance Mutation Frequency (TMF) of CSC was more than 3 times higher than that of the solvent control at higher concentrations (80-150 μg/ml), and induced dose-related mutagenic effects in LS178Y cells. There were no mutagenic effects of TK gene appeared in the SCs and ECEs in the dose range.

Infants are exposed to environmental chemicals via many routes including ingestion of breast milk. Persistent organic pesticides are environmental chemicals that are found in measurable concentrations in human populations and biological media even though some of these chemicals have been phased out of commerce, production, or release to the environment. There are few studies quantifying human milk persistent organic pesticides especially in US populations and even fewer studies exist with repeated measures over time from the same individual. The US EPA Methods Advancement for Milk Analysis (MAMA) study developed and adapted methods to measure persistent organic pesticides and other environmental chemicals in milk and serum twice during lactation (6-27 weeks and 3-4 months postpartum) in 34 North Carolina women. Milk and serum were quantified for persistent organic pesticides (hexachlorobenzene (HCB), hexachlorocyclohexane (HCHC), oxychlordane, trans-nonachlor, DDE, DDT and Mirex). A majority of both the milk and serum pesticide samples contained pesticides at concentrations above limit of detection (LOD), excluding serum HCHC. The majority of the chemicals did not show depletion (decreased amount of chemical between two points in lactation) in milk between visits; only HCB showed decreased concentrations in milk at the second clinic visit. Mean concentrations were highest for p,p′-DDE (163 ng/ml lipid in milk, 102 ng/ml lipid in serum) with Mirex having among the lowest concentrations (0.7 ng/ml lipid in milk, 102 ng/ml lipid in serum) with Mirex having among the lowest concentrations (0.7 ng/ml lipid in milk, 102 ng/ml lipid in serum). These data suggest that milk is a viable biomarker for downstream application in early healthcare intervention for military working dogs with occupational chemical exposures.
Exposure to polychlorinated biphenyls (PCBs) can occur through multiple routes and sources, including dietary intake, inhalation, dermal contact, and ingestion of dust and soils. Dietary exposure to PCBs is often considered the primary exposure route for the general population, however, recent studies suggest an increasing contribution from indoor inhalation exposure. Here, we estimated the relative contribution of different PCB exposure pathways for the general population and for select age groups. We conducted a targeted literature review of PCB concentrations in environmental media, as well as of total dietary intake. Using the average concentrations from the studies identified, we estimated PCB exposure through different routes for the general population. In addition, we assessed exposure via environmental media for select age groups. We identified a total of 70 studies, 64 that provided background PCB concentrations for one or more of the environmental media of interest and 6 studies that provided estimates of total dietary intake. Using estimates from these studies, dietary intake of PCBs was the major exposure pathway for the general population. Overall, however, our review identifies important limitations in the data available to assess population exposures, highlighting the need for more current and population-based estimates of PCB exposure, particularly for indoor air and dietary intake.

**2638 A Review of Polychlorinated Biphenyl Concentrations in Environmental Media Worldwide: Relative Contributions of Exposure Routes**


**2639 Ex Priori: A Screening-Level Chemical Prioritization Dashboard for Consumer Exposures**


*If these are the things I do and the products I use, then to which chemicals am I being exposed?* Exposure to a wide range of chemicals through our daily interaction with consumer products is ubiquitous and largely unavoidable. Due to the breadth of consumer product formulations of chemical ingredients and the diversity of consumer-specific habits and practices the task of quantifying one’s chemical exposure profile can be overwhelming. The US EPA’s Exposure Prioritization (Ex Priori) tool is a simplified, quantitatively visual dashboard that makes use of data from various inputs to provide rank-ordered prioritization for dose-metric and to address the very question of one’s personal chemical exposure to all chemicals in consumer products. Developed as a screening level flexible dashboard-type chemical prioritization tool, Ex Priori provides a scenario-specific ranking of multiple chemical exposures from consumer products while accounting for physico-chemical properties (e.g., partitioning coefficients); exposure factors; and variations in product formulations, activity patterns, and product use profiles (e.g. habits and practices). Additionally, Ex Priori extends traditional exposure models by incorporating chemical-specific predictions of ADME processes to estimate route- and chemical-specific internal doses. Ultimately, Ex Priori translates personal chemical exposure from seven exposure pathways for 1634 chemicals present in 228 consumer product categories to a rank-ordered interactive visual representation of body burden. Finally, Ex Priori contains customizable controls (i.e., sliders) to vary user-specific activity which can be updated by the user to account for particular exposures such as hobbyist or occupational users who have higher exposure potential. By adjusting Ex Priori controls for different scenarios, all 1634 consumer product ingredients are simultaneously re prioritized on anticipated body-burden, enabling a fast representation of one’s day-to-day personal multi-chemical exposures from consumer products. This abstract does not necessarily represent the views or policies of the US Environmental Protection Agency.

**2640 Biomonitoring of Mycotoxins in Human Hair as Novel Matrix to Assess Cumulative Exposure**


The analysis of human urine and plasma samples represents a precise approach in order to assess the exposure to mycotoxins. Nevertheless, these matrices can only provide information about short-to-medium-term exposure due to stability issues concerning the biomarkers. In this line, hair emerges as a promising matrix for long-term exposure assessment studies, since contaminants remain embedded in the keratin matrix or covalently linked to melanin, supplying high stability, whereas its collection implies a non-invasive procedure. Considering a standard growth-rate of 1 cm/month, the exposure can also be evaluated during different time frames, adding another feature to hair analysis. Therefore, the aim of this work was to evaluate the presence of nine toxicologically relevant mycotoxins in 50 human hair samples through the capability offered by the high performance liquid chromatography (HPLC) coupled to Q-TOF high resolution mass spectrometry (HRMS) technique. To achieve this, hair samples were initially washed with a non-ionic detergent and distilled water and later subjected to enzymatic digestion with Pronase E alongside the redox reagent diethiothreitol (DTT), for reducing disulfide bonds within the keratin matrix. The digested samples were then extracted using a QuEChERS-based extraction. Mycotoxins from main important toxigenic fungi including aflatoxins (AF) B1, B2, G1 and G2, zearalenone (ZEN), T-2 toxin (T-2), alternariol (AOH) and sterigmatocystin (STE) were covered within this study due to their relevance data for hair of our study population. After spiking experiments at 0.5, 5 and 50 ng/g were obtained, all measurements with acceptable precision. Preliminary results of the biomonitoring study reflect contamination with AFB2, CIT, ZEN, T-2 and AOH, occurring in most cases at low levels of ng/g. The presence of stringently regulated mycotoxins could point to a cumulative exposure that could have been underestimated considering toxicokinetic parameters. The results highlight hair as a suitable matrix for biomonitoring-based exposure assessment studies and support further investigations for the development of biomarkers.

**2641 Determining Dietary Exposure to Glyphosate Resulting from Recommended US Diets**


Concerns regarding exposure to glyphosate have been raised following the International Agency for Research on Cancer’s (IARC) classification of the compound as “probably carcinogenic to humans.” While there is no consensus among the scientific community as to its carcinogenicity, dietary intake is a major route of exposure to glyphosate in the general population. It has been noted that particular foods may contain higher glyphosate residues than others. As such, we investigated whether certain diets are associated with increased intake of glyphosate. Dietary glyphosate intake was estimated for three diets commonly followed in the U.S. at various calorie levels, as prescribed by the U.S. Department of Agriculture (USDA). To determine the total glyphosate exposure associated with each diet, we utilized glyphosate residues reported in a market survey of 22 dairy/meat products, 220 fruit/vegetable products, and over 110 types of grain/pulse/soy products (n=7,955). These exposures were compared to a proposed health-based regulatory benchmark to determine potential cancer risk. When detected, the median glyphosate ingestion for Vegetarian, U.S.-Style, and Mediterranean diets ranged from 0.00536 to 0.03612, 0.00605 to 0.03620, and 0.00608 to 0.03697 μg/day/calorie, respectively. The 95th percentile glyphosate ingestion for the respective diets ranged from 0.01939 to 0.55405, 0.01906 to 0.56591, and 0.01930 to 0.61067 μg/day/calorie. No considerable difference in glyphosate intake was observed between the three diets. Rather, dietary exposure to glyphosate is primarily based on the quantity of high residue food ingested. Based on our assessment of caloric intake of 1800 to 2800 calories/day, median exposure for each of the three diets was greater than the OEHHA’s proposed No Significant Risk Level (NSRL) of 1100 μg/day for glyphosate. Although the majority of 95th percentile exposures are below the NSRL for 1,800 to 2,800 calories/day for each diet, the benchmark may be exceeded if a diet includes a large quantity of high residue foods. Additionally, a well-balanced diet comprised of a variety of foods from each food group, the cancer risk from glyphosate exposure from common dietary styles is unlikely to be of concern in the U.S.

**2642 Characterization of Particle Size Distribution and Number Concentration of Nano Objects Generated during Synthesis of Silver and Gold Nanoparticles**


Engineered nanomaterials (ENMs) of at least one particle diameter of between 1-100 nm are being implemented in medical and industrial applications due to their physical and chemical properties. During the synthesis of ENMs various occupational exposures occurs, leading to severe health adverse effects. However, to date, there are few exposure assessment studies focused on the
ENMs synthesis process. The study aimed to assess exposure to gold and silver nanoparticles during the synthesis process. Nanoparticles were monitored in a synthesis laboratory where gold and silver particles were synthesized. Data was monitored using a scanning mobility particle sizer (SMPS) over the entire synthesis period for both nanoparticle syntheses. Each synthesis period took approximately 1-hour duration. Each exposure scenario was monitored three times in order to reduce the uncertainty in the data outputs. Furthermore, a time-weighted average concentration was calculated and compared with the occupational exposure limit (OEL) for silver nanoparticles and the proposed OEL for gold nanoparticles. During synthesis of silver nanoparticle, 8-hour time-weighted average (TWA) was calculated to be 0.09 µg/m³, which is lower than the proposed occupational exposure limit of 0.15 µg/m³. Currently, gold nanoparticles do not have dedicated exposure limit, however, there is a proposed occupational exposure limits of 20 000 particle/m³ which was found to be higher than the calculated TWA for gold nanoparticles at 1728.01 particle/m³. The TWA-8 hour calculated for both silver and gold nanoparticles shows that the exposure level is relatively lower than the stipulated and proposed OEL’s, respectively. The major conclusion drawn from this research is that exposure to nanoparticles in Nano-synthesis laboratories may potentially be kept below the exposure risk level with the use of engineering controls (LEV) before considering personal protective equipment (PPE).

2644 Informal Workers Exposed to Potentially Toxic Elements and Risk for COVID-19 Infection

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Welders occupationally exposed to Potentially Toxic Elements (PTE) (n=26) and a control group (n=25) were followed by an online questionnaire throughout four months during the COVID-19 pandemic (June to September) to characterize symptoms and preventive behaviors. The exposed group performed informal home-based work at an important Brazilian productive chain neighborhood but do not work with chemical exposure. A previous study on the productive profile found high immunity problems in the group exposed to PTE. Before the pandemic, participants were assessed regarding blood PTE levels (using Inductively Coupled Plasma Mass Spectrometer). Elements such as Lead (Pb), Cadmium (Cd), and Arsenic (As) were found significantly higher for the welders (Pb: 1.88 µg/dL, 95%CI: 1.54 - 2.28; Cd: 0.21 µg/L, 95%CI: 0.13 - 0.34; As: 0.44 µg/L, 95%CI: 0.39 - 0.50) compared to the control group (Pb: 1.04 µg/dL, 95%CI: 0.90 - 1.19; Cd: 0.01 µg/L, 95%CI: 0.01 - 0.02; As: 0.35 µg/L, 95%CI: 0.28 - 0.44); p<0.001 for all comparisons. The adherence to social distancing kept high between the workers (>83%) throughout the follow up while continuously decreased in the control group (from 86% initially to 63% at the last evaluation). Eight participants reported had tested for COVID-19: 6 results were positive, 5 of them of individuals from the control group (p=0.09). We assessed the incidence of a composite outcome defined for the presence of at least a symptom suggestive of COVID-19 infection (cough, congestion, sore throat, loss of taste or smell), and fever) during repeated evaluations. We analyzed associations using random-effect logistic regression considering the individual as the clustering variable. Throughout the four months, we obtained 165 observations from the 51 participants and identified 54 symptomatic episodes. Despite not significant, exposed workers had an incidence rate ratio (IRR) higher than the non-exposed ones: IRR=1.04 (95%CI 0.72 - 1.72). The symptomatic events were also not associated with other variables evaluated, such as age, social distancing, or PTE blood levels. Although evidence regarding immune problems due to PTE exposure and the blood PTE levels found higher for the welders, we did not find a significant association with symptomatic episodes suggestive of COVID-19. This study was funded by FAPESP (2018/18391-0) and CNPq (140192/2019-0 and 312655/2019-0).

2645 Physical and Chemical Characterization of Tire and Road Wear Particles: Implications for Single Particle Analysis

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Tire and road wear particles (TRWP) are generated from friction between the tire and the road. The fate of TRWP depends on both the mass concentration as well as individual particle characteristics, such as particle diameter and density, similar to other environmentally dispersed microplastic particles (MP). TRWP are comprised of complex mixtures of microplastics, pavement encrustations and the identification of an individual TRWP in environmental samples has been limited by characteristics which interfere with the spectroscopic techniques most often used in MP research (e.g., Raman microscopy). The purpose of this research was to apply suitable analytical techniques to characterize the specific physical and chemical properties of individual TRWP to better develop methods for single particle analysis in environmental samples. To this end, scanning electron microscopy coupled with energy dispersive X-ray spectroscopy (SEM/EDX) mapping and time-of-flight secondary ion mass spectrometry (ToF-SIMS) mapping were utilized to generate detailed elemental and organic surface maps for various tread-containing material including TRWP. TRWP were characterized as (1) elongated/round shape with variable amounts of mineral encrustation, (2) elemental surface characteristics including co-localization of (Si,Zn)Na+ (Si, K, Mg, Ca, and Al), and (3) co-localization of organic surface markers, such as CH2 and C3H5. Addition of a density separation step (<2.2 g/cm³) to the single particle analysis techniques allowed for the determination of average primary TRWP particle size (34 µm by number distribution and 49 µm by volume distribution) and aspect ratio (65% of TRWP with an aspect ratio >1.5). Taken together, the use of chemical mapping techniques such as SEM/EDX and ToF-SIMS can support future research efforts that aim to identify complex MP and further advance our knowledge on environmental fate and impact.

2646 The Effects of Incorporating Nonlinearity in LCA: Characterizing the Impact on Human Health

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Processes that govern environmental mechanisms including fate, transport, and exposure are generally non-linear. Characterization models in life cycle impact assessment (LCA), however, often linearize such processes, while the implications of linearizing non-linear processes have not been fully understood. Recently, non-linear models have been incorporated into characterization modeling, allowing the opportunity for a comparison. Here, we test potential effects of incorporating three types of non-linear processes into life cycle assessment (LCA): emission rate, environmental fate, and exposure-response. We compare the characterized results of human health impact due to (1) USEtox, a conventional, steady-state model and (2) the CLiCC suite, which employs dynamic emission profiles, non-steady state fate and transport, and no-effect exposure thresholds. Under constant emission rates, the two approaches display comparable results over a long period of time. When significant temporal variations are introduced to emission rates, however, the results from the two approaches start to deviate. On the one hand, pulse emissions averaged over time tend to show lower human health impacts under USEtox as compared to CLiCC suite, as the level of exposure...
sure shortly after the pulse emission temporarily shifts the dose-response regime to a steeper tendency in the curve. On the other hand, USEtox, in the absence of no-effect exposure thresholds, tends to show higher human health impacts compared to CLiCC suite for low-level emissions with little temporal variation. Our results call for a careful interpretation of characterized results, especially when the emissions are known to exhibit large temporal variations.

They provide insight into PCBs potential modes of action. The roles of estrogenic PCBs and mire-122-Sp in environmental liver disease warrant further investigation. miRNA-derived ‘liquid liver biopsy’ appears to be a useful tool for environmental hepatology cohort studies. This does not necessarily reflect USEPA or ATSDR policy.

2647 Application of Systematic Evidence Mapping to Characterize Baseline Legacy Chemical Contamination: An Urban Estuary Case Study

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Natural disasters (i.e. floods, hurricanes, wind, excessive rainfall, etc.) impact urbanized estuarine environments by dispersing sediment bed loads, potentially posing an environmental and public health risk due to the presence of legacy or emerging chemicals contamination. However, it is challenging to characterize the baseline spatial and temporal distribution of environmental chemical contamination prior to natural disasters. To address this gap, we propose the application of a systematic evidence mapping to comprehensively integrate available data from diverse sources within an urbanized Texas estuary: Galveston Bay and the Houston Ship Channel (GB/HSC). The objective of this systematic evidence map is to determine whether a baseline historical spatial and temporal distribution dataset of legacy chemical contaminants is available in the study area. We developed inclusion/exclusion criteria using a Condition, Context, Population (CoCoPop) statement that addresses five areas: chemicals of interest (condition), geographic region and environmental descriptor (context), sediments (population), and study design. In total, our search identified 487 studies with 55 studies included after title/abstract screening. 24 studies were analyzed after full text extraction. Most of the studies reported dioxins/furans or mercury within the Houston Ship Channel, with limited reporting of other organics and metals (i.e. PAHs, PCBs, Pb, Zn, etc.). Reported metals were commonly reported in Lower Galveston Bay, while organics were reported in the Houston Ship Channel. Sampling frequency also varied and was inconsistent between studies. Our systematic evidence map revealed that within GB/HSC, dioxins/furans and mercury are significant legacy chemical contaminants. Furthermore, the reported chemical concentration data are sparsely and samples were collected inconsistently. Thus, it is difficult to determine the spatial and temporal distribution of baseline levels of legacy chemicals. To further aid risk evaluation of sediments redistributed after natural disaster, future published studies can improve both their keyword indexing and their environmental data reporting of geocoordinates and individual chemical concentrations.

2648 Circulating microRNAs Are Associated with PCB Exposures and Liver Disease in the Anniston Community Health Survey

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PCB exposures have been associated with liver disease in cohort studies and steatohepatitis with liver necrosis in animal models. MicroRNAs (miRNAs) are non-coding RNAs that maintain cellular homeostasis, possibly in response to environmental exposures. This study tests the hypothesis that PCB-associated liver necrosis (suspected toxicant associated steatohepatitis) is associated with circulating miRNAs in an exposed human cohort. IRB approval was obtained for this analysis of 738 participants in the cross-sectional Anniston Community Health Survey (ACHS). Necrotic liver diseases were defined by a combination of serum keratin 18 (K18) biomarkers as published (PM60166463). Here, association studies were performed between highly expressed serum hepatotoxicity miRNAs (n=35) and categorical liver disease (primary endpoint); continuous serum PCBs (n=35), cytokines (n=4), and HOMA-IR. Ingenuity Pathway Analysis (IPA) was performed. The necrotic liver disease category (n=359/738) was associated with 4 up-regulated miRNAs (miR-99a-5p, miR-122-5p, miR-192-5p, and miR-320a) and 5 down-regulated miRNAs (let-7d-5p, miR-17-5p, miR-24-3p, miR-1973p, and miR-221-3p). 11 miRs were associated with 24 PCBs, most commonly congers with (anti-)estrogenic activity. 22 miRNAs were associated with continuous K18. Most of the exposure-associated miRNAs were also associated with at least one hepatocyte death, pro-inflammatory cytokine and/or insulin resistance biomarker. miR-122-5p was the miRNA most significantly associated with each of the exposure and disease biomarkers. IBA demonstrated enrichment in liver toxicity functions related to inflammation/hepatitis, hyperplasia/hyperproliferated estuarine environment, and other hepatic cell death. P36 was confirmed as the miRNA-associated networks. These results support the hepatotoxicity of PCBs in humans and previously reported high liver disease prevalence in ACHS.

2649 Bayesian Inference of Chemical Exposures from NHANES Urinary Biomonitoring Data

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Biomonitoring data provides information about the chemicals commonly present in humans, and their concentrations. However, it is also important to understand which chemicals might be posing an environmental risk to the generation of observed transformation product biomarkers, or metabolites, for the sake of estimating exposure and risk to public health. Here, we used Bayesian methodology to infer ranges of exposure to parent compounds consistent with urinary biomarker levels measured by the U.S. Centers for Disease Control and Prevention in its nationally representative National Health and Nutrition Examination Survey (NHANES). Metabolites were linked to likely parent chemicals using information from the NHANES reports and text mining of PubMed abstracts for metabolite names and synonyms. This research produced 209 links between the 106 NHANES metabolites (data spanning from 1999 to 2016) and 569 candidate parent chemicals. We estimated chemical risk among various demographic groups, including the 0-5 year age range that debuted in the 2015-2016 phase (measurement data for 50 metabolites). Additionally, we identified temporal trends for parent chemicals by comparing exposure and risk across multiple NHANES phases individually. A number of the chemicals with the largest change in predicted exposure, around a 2-fold increase, are insecticide permethrin metam (which share the metabolite 3-phenoxbenzoic acid; diazion and coumaphos, which share the metabolite diethylphosphate). These inferred exposure intake rates, when coupled with toxic doses predicted by US EPA’s TEST tool and high throughput toxicokinetic, allow the identification of public health priority chemicals via risk-based bioactivity:exposure ratios (BER). Here, our chemical BER estimates ranged from 3x10^-4 to 4x10^-6, indicating a 5-fold variation in risk across parent chemicals. The tools and methods used to create these analyses have been thoroughly documented in preparation for peer-review and public distribution to the biomonitoring community. This abstract does not necessarily reflect USEPA policy.

2650 Informatics-Based Approaches for Collecting and Curating Consumer Product Data for Exposure Assessments


Quantitative data on product chemical composition is necessary for characterizing consumer exposure to chemicals. EPA’s Office of Research and Development (ORD) has built models, such as the High-Throughput Stochastic Human Exposure and Dose Simulation model (SHEDS-HT), that use this data to estimate exposures for over 300 hierarchically harmonized product use categories (PUCs). However, this data is often lacking or in various formats, making it difficult for model use. To fill this data need, ORD has developed automated approaches for curating data on thousands of products and chemicals from public documents (safety data sheets, manufacturer ingredient disclosures, ingredient lists). However, curation of products to PUCs is historically a bottleneck, requiring manual assessment of product names. Here, we use natural language processing machine learning approaches to hasten this curation step. The model training dataset was composed of all products within ORD’s Chemical and Products Database (CPDat) with a manually assigned PUC; models were built for PUCs with at least 30 products (63,593 products; 161 PUCs). For modeling, each product-brand name was combined, cleaned, lemmatized to word roots, and converted to a quantitative vector using standard libraries. A Support Vector Machine (SVM) classifier was created for each level of the 3+ tier PUC classification, each informed by the higher tier prediction. The probabilistic SVM models were used to generate multiple predictions per tier; the median predicted PUC was selected. Five-fold cross validation was performed (stratified by PUC to ensure proportional representation) resulting in an average 94% classification accuracy. The expanded data were used to update consumer exposure predictions and characterizing BAS, using SHEDS-HT, which provided refined aggregate and PUC-specific consumer exposure distributions, particularly for home care and home maintenance PUCs (previously data poor in terms of products in CPDat). In summary,
implementation of informatics approaches for managing and curating public documents are rapidly expanding the quantity and quality of data available for assessing consumer exposure to chemicals in consumer products. This abstract does not necessarily reflect US EPA policy.

**2651 Expansion and Refinement of Chemical Use Data for Characterizing Exposure Pathways**

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The US EPA is responsible for evaluating thousands of chemicals for the potential risks they may pose to humans and ecosystems, which necessitates information on hazard and exposure potential for each chemical. To support chemical risk assessment, EPA’s Office of Research and Development (ORD) must identify and characterize relevant exposure pathways - the path of a chemical from a source to a receptor. How a chemical is used (e.g. in a consumer, occupational, or industrial context) is critical to determining exposure pathways. ORD has developed a data management and curation application, called Factotum, which facilitates the rapid collection and distribution of high-quality chemical and exposure related data from public documents. Within Factotum, there has been a significant focus on chemical composition of consumer products, functional role of chemicals within products and processes, and presence of chemicals on reported specific or general use lists. We hypothesized that pre-treatment with biologically relevant concentrations of nicotine prior to mancozeb exposure would lead to neuroprotection and supports the hypothesis that nicotine has a neuroprotective role in reducing DAergic damage induced by agriculturally relevant exposures of the toxicant manzate. Future studies will utilize mutants and biochemical assays to elucidate mechanism of protection and explore potential therapeutic effects of nicotine in neurodegeneration.

**2653 Chlorpyrifos and Chlorpyrifos-Oxon Produce Dopaminergic Toxicity in C. elegans**

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Chlorpyrifos (CPF) is an organophosphate pesticide still in use in the United States of America while banned in many countries. Neurotoxicity has been primarily attributed to the inhibition of acetylcholinesterase (AChE). More recently, data suggest that CPF may also produce dopaminergic neurotoxicity. Here, we aimed to test the hypothesis that CPF would produce selective neurotoxicity in dopaminergic neurons in C. elegans. Worms were treated with CPF at 0.156 – 500 µM for 48 - 72 hrs. At 50 µM – 500 µM, we observed clear evidence of neurodegeneration in dopaminergic neurons, decreased dopamine-dependent behavior, and decreased mitochondrial staining, along with developmental defects. In line with the observed developmental defects, a variation in neurotoxicity was also observed specific to treatment at different larval stages. The use of antioxidant regimens such as N-acetyl cysteine and hydroxybutyric acid failed to ameliorate neuronal loss, suggesting broad oxidative stress may not be a primary mechanism of neurotoxic action. Washout experiments to compare CPF neurotoxicity on acetylcholinergic and dopaminergic function indicated that acetylcholinergic neurotoxicity was reversible, while dopaminergic toxicity was irreversible. Moreover, dopaminergic neurotransmission was affected at lower doses than the acetylcholinergic system. Taken together, these findings suggest that dopaminergic neurons exhibit heightened sensitivity relative to other neuronal cell types. Next, we studied the effect of the toxic CPF metabolite, chlorpyrifos oxon (CPF-O), which was found to be more toxic in terms of both cell loss and function relative to CPF. Preliminary experiments suggest that CPF and CPF-oxon inhibit mitochondrial complex II. Interestingly, this inhibition was more prominent in the case of CPF, suggesting other mechanisms may underlie the increased potency of CPF-oxon, such as inhibition of dopamine catabolism, a known mechanism of CPF neurotoxicity. Our findings support for CPF as a dopaminergic neurotoxicant and also suggest some specific mechanistic pathways that should be further explored.

**2652 Investigation of the Protective Effects of Nicotine in Pesticide-Induced Neurodegeneration in the Model Organism C. elegans**

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Although tobacco use has long been linked with increased risk of cancer, epidemiological studies have also linked tobacco use, especially nicotine, to reduced risk of Parkinson’s disease, a neurodegenerative disease characterized by the loss of dopaminergic neuron (DAergic) function. Nicotine has higher binding affinity than the normal substrate acetylcholine to nicotinic acetylcholine receptors (nAChRs) in DAergic neurons, resulting in neuronal stimulation. Previous studies have indicated that chronic exposure to environmental toxicants, particularly the fungicide mancozeb, can lead to degeneration of DAergic neurons in the model organism Caenorhabditis elegans (C. elegans). We hypothesized that pre-treatment with biologically relevant concentrations of nicotine prior to mancozeb exposure would lead to neuroprotection in C. elegans. Wild type N2 nematodes were pre-treated with nicotine and then treated chronically with mancozeb (dual paradigm). Behavioral assays that target DAergic neurons (nose touch) show a statistically significant decrease in response for mancozeb treatment compared to control (p<0.0005) and no difference in assays targeting other neurons. Nicotine, either alone or dual treatment, showed do difference to control suggesting that DAergic stimulation by nicotine is neuroprotective. C. elegans with green fluorescent protein (GFP) tagged dopamine transporter (BZ555) were used to assess DAergic health. Healthy DAergic neurons fluoresce at higher levels than damaged or non-functional neurons. Fluorescence following nicotine treatment was significantly different compared to control (p<0.05) indicating increased DAergic activity. When comparing mancozeb to control, there was a trend toward decreased fluorescence. Additionally, there was a statistical difference when comparing mancozeb and nicotine treatments (p<0.0005) and no difference with dual treatment to control, supporting an antagonistic role of nicotine in mancozeb induced neurodegeneration. Taken as a whole the data supports the hypothesis that nicotine has a neuroprotective role in reducing DAergic damage induced by agriculturally relevant exposures of the toxicant manzate. Future studies will utilize mutants and biochemical assays to elucidate mechanism of protection and explore potential therapeutic effects of nicotine in neurodegeneration.

**2654 Modeling ALS in a Mutant Zebrafish Expressing a TDP-43 Acetylation Mimic**

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease that targets and progressively motor neurons, leading to muscle atrophy, paralysis, organ system failure, and ultimately death within five years in 80% of cases. A pathological hallmark of ALS is the presence of cytoplasmic aggregates of TAR DNA binding protein (TARDBP, TDP-43), which is important in RNA splicing, and regulation of mRNA translation. While mutations in the glia-rich C-terminus is known to be associated with familial ALS, TDP-43 aggregates occur at high frequency in sporadic forms that are not linked to identified mutations. Recently, a human cell line with a K145Q mutation within an amyotrophic lateral sclerosis (ALS). Generating the mutant on this transgenic background facilitates the expansion of different pathways - the path of a chemical from a source to a receptor. How a chemical is used (e.g. in a consumer, occupational, or industrial context) is critical to determining exposure pathways. ORD has developed a data management and curation application, called Factotum, which facilitates the rapid collection and distribution of high-quality chemical and exposure related data from public documents. Within Factotum, there has been a significant focus on chemical composition of consumer products, functional role of chemicals within products and processes, and presence of chemicals on reported specific or general use lists. We hypothesized that pre-treatment with biologically relevant concentrations of nicotine prior to mancozeb exposure would lead to neuroprotection and supports the hypothesis that nicotine has a neuroprotective role in reducing DAergic damage induced by agriculturally relevant exposures of the toxicant manzate. Future studies will utilize mutants and biochemical assays to elucidate mechanism of protection and explore potential therapeutic effects of nicotine in neurodegeneration.

**2651 Expansion and Refinement of Chemical Use Data for Characterizing Exposure Pathways**

S. Handa1, J. T. Wall1, K. Isaacs1, A. Larger2, B. Horton2, D. Lyons1, and K. Dionisio1. 1US EPA ORD, Research Triangle Park, NC; and 2Oak Ridge Associated Universities, Research Triangle Park, NC. Sponsor: J. Wambaugh

The US EPA is responsible for evaluating thousands of chemicals for the potential risks they may pose to humans and ecosystems, which necessitates information on hazard and exposure potential for each chemical. To support chemical risk assessment, EPA’s Office of Research and Development (ORD) must identify and characterize relevant exposure pathways - the path of a chemical from a source to a receptor. How a chemical is used (e.g. in a consumer, occupational, or industrial context) is critical to determining exposure pathways. ORD has developed a data management and curation application, called Factotum, which facilitates the rapid collection and distribution of high-quality chemical and exposure related data from public documents. Within Factotum, there has been a significant focus on chemical composition of consumer products, functional role of chemicals within products and processes, and presence of chemicals on reported specific or general use lists. We hypothesized that pre-treatment with biologically relevant concentrations of nicotine prior to mancozeb exposure would lead to neuroprotection and supports the hypothesis that nicotine has a neuroprotective role in reducing DAergic damage induced by agriculturally relevant exposures of the toxicant manzate. Future studies will utilize mutants and biochemical assays to elucidate mechanism of protection and explore potential therapeutic effects of nicotine in neurodegeneration.
Alzheimer's disease (AD) is a neurodegenerative disease characterized by the accumulation of amyloid beta plaques, neurofibrillary tangles, and cognitive decline. Despite the significant public health crisis that it poses, there is no clinically available disease-modifying therapy. Thus, it is critical to identify modifiable risk factors that could decrease the risk of disease or slow the disease course. Several studies have suggested that high levels of meat consumption may increase AD risk, highlighting the diet's role in AD. 2-aminopyridine (PhIP) is a prevalent dietary heterocyclic aromatic amine (HAA) formed during high temperature meat cooking. Recent work from our lab has shown that PhIP exposure in mice induced AD-relevant neuropathology. Protein aggregation is a common feature of many neurodegenerative diseases; the aggregation process is believed to be central to AD pathogenesis. HAAs are highly reactive molecules, capable of promoting protein aggregation. In the present study, we tested the hypothesis that PhIP and one of its major reactive metabolites, N-OH-PhIP, would promote aggregation of the critical AD protein tau. Primary cortical neurons, SH-SYSY cells overexpressing amyloid precursor protein isoform (APP wild type and APP mutant), and isolated mitochondria were used to identify mechanisms associated with AD-relevant neurotoxicity induced by HAAs. HAAs increased APP expression in SH-SYSY cells and BACE1 expression in primary neuronal cells. In cell-free systems, preliminary data indicate that HAAs directly promoted the fibrillization of tau, and N-OH-PhIP was more potent in inducing tau aggregation. HAAs also induced tau pathology in primary neuronal cells. SH-SYSY and primary neuronal cells treated with HAAs exhibited altered mitochondrial content and levels of mitophagy related proteins. N-OH-PhIP decreased mitochondrial function in primary neuronal cells and the activity of mitochondrial complex III in mitochondria. Our study indicates that HAAs induce AD-relevant neurotoxicity by directly promoting biophysical interactions (aggregation) of AD-relevant proteins and through effects on mitochondria. Collectively, our study suggests a potential link between diets high in HAAs and AD.

Neuroinflammation is implicated in the progression and pathology of several neurodegenerative diseases including Alzheimer's disease (AD). While AD presents differently in individuals, advancing age, female sex and presence of the strongest known genetic risk factor, APOE4 (E4) genotype, have been shown to contribute greatly to the increased risk of AD. APOE is predominantly expressed in astrocytes and microglia. These E4 glia from humans and mice have been shown to express a more reactive phenotype compared to APOE3 (E3). We hypothesized that age, sex and APOE genotype modify the response to an inflammatory stimulus, potentially by inducing proinflammatory cytokine production and secretion in a cell-type, sex, and genotype-specific manner. We first sought to define the effects of an inflammatory stimulus on sex-specific E3 and E4 primary microglia (PMG) and astrocytes (PMA). Our findings indicate that both male and female E4 PMG produced at least a 65% increase in media nitrite levels than E3 PMG (p<0.001). Additionally, a further increase of 25% was observed in E4 females compared to E4 males. PMA from E4 females produced 50% more media nitrite compared to E3 females. To investigate in vivo, male and female humanized targeted replacement E3 and E4 mice at 3 or 16 months of age were injected with lipopolysaccharide (LPS, 0.5 mg/kg) and sacrificed 4h later. LPS induced a higher expression of Il1b and Tnf mRNA in the frontal cortex and hippocampus of young and aged E4 mice compared to E3. Il1b expression increased in the hippocampus by ~30-fold and ~40-fold in aged E4 males and females, respectively. In contrast, Il1b only increased ~15-fold in aged E3 males and females. Similar effects were observed in Tnf and Il6 expression in the hippocampus (p<0.001). In the young cohort, no sex differences were observed, but Il1b and Il6 gene expression in E4 males and females increased by 2-fold compared to E3. These data indicate that a peripheral LPS challenge induces a higher increase in proinflammatory cytokine mRNA expression in older E4 mice and this effect appears to be sex and region-specific. Together, these data demonstrate that multiple factors contribute to susceptibility to neuroinflammation and provide insight into the role of age, sex and genotype in this susceptibility. Supported in part by NIH R01ES026057 and NIH R01ES024288.

Alzheimer's disease (AD) is the leading cause of dementia in the United States. With over 5 million Americans affected, it is the 6th leading cause of death in the nation. There is increasing evidence that the environment plays a potential role in the etiology of the disease. We have previously reported that AD patients had a significantly higher serum level of dichlorophenyl dichloroethene (DDE), the metabolite of dichlorophenyl dichloroethylene (DDT), compared to age-matched controls. It has also been suggested that the environmental pollutant lead (Pb) is a risk factor for AD through experimental studies in non-human primates and rodents. Both Pb and DDT have individually been shown to increase levels of amyloid precursor protein (APP) and amyloid β (Aβ), which are hallmark characteristics of AD. Since these pollutants were highly prevalent in the 20th century, populations exposed are reaching ages where AD risk is at its highest (>65 years of age). We sought to determine whether Pb and DDT may interact to produce greater effects in the amyloid pathway. To explore this, we utilized HT22 cells which are an immortalized mouse hippocampal cell line. Exposing undifferentiated HT22 cells to Pb, DDT, or a combination of both did not change APP or Aβ levels as quantified by immunofluorescence staining. Differentiating HT22 cells with N2 supplement has been previously shown to induce post-mitotic cholinergic cells. Upon differentiation of HT22 cells we observed significant increases in APP levels after 24 hours of treatment; 1 μM DDT (63%), 10 μM Pb (89%), and combination (257%) as compared to control. We also observed greater increases after 48 hours of treatment; 1 μM DDT (159%), 10 μM Pb (160%), and combination (257%) as compared to control. To recapitulate our results, we utilized primary mouse hippocampal neurons isolated from C57BL/6J postnatal day 0-1 mouse pups. APP levels increased by 40%, 60%, and 74% in primary hippocampal neurons treated with 1 μM DDT, 10 μM Pb, and the combination, respectively. Together, these data demonstrate that Pb and DDT play a role in the amyloid pathway, potentially providing for a new understanding of pesticide and heavy metal implications in AD. Supported in part by NIH R01ES026057.

**Ozone Exposure in a Mouse Model of Alzheimer's Disease: Evidence for a Bi-directional Lung-Brain Axis**


An increasing body of epidemiological evidence implicates components of urban air pollution, including ozone (O3), as a risk factor for Alzheimer's disease (AD). With over 5 million Americans affected, it is the 6th leading cause of death in the nation. There is increasing evidence that the environment plays a potential role in the etiology of the disease. We have previously reported that AD patients had a significantly higher serum level of dichlorophenyl dichloroethene (DDE), the metabolite of dichlorophenyl dichloroethylene (DDT), compared to age-matched controls. It has also been suggested that the environmental pollutant lead (Pb) is a risk factor for AD through experimental studies in non-human primates and rodents. Both Pb and DDT have individually been shown to increase levels of amyloid precursor protein (APP) and amyloid β (Aβ), which are hallmark characteristics of AD. Since these pollutants were highly prevalent in the 20th century, populations exposed are reaching ages where AD risk is at its highest (>65 years of age). We sought to determine whether Pb and DDT may interact to produce greater effects in the amyloid pathway. To explore this, we utilized HT22 cells which are an immortalized mouse hippocampal cell line. Exposing undifferentiated HT22 cells to Pb, DDT, or a combination of both did not change APP or Aβ levels as quantified by immunofluorescence staining. Differentiating HT22 cells with N2 supplement has been previously shown to induce post-mitotic cholinergic cells. Upon differentiation of HT22 cells we observed significant increases in APP levels after 24 hours of treatment; 1 μM DDT (63%), 10 μM Pb (89%), and combination (257%) as compared to control. We also observed greater increases after 48 hours of treatment; 1 μM DDT (159%), 10 μM Pb (160%), and combination (257%) as compared to control. To recapitulate our results, we utilized primary mouse hippocampal neurons isolated from C57BL/6J postnatal day 0-1 mouse pups. APP levels increased by 40%, 60%, and 74% in primary hippocampal neurons treated with 1 μM DDT, 10 μM Pb, and the combination, respectively. Together, these data demonstrate that Pb and DDT play a role in the amyloid pathway, potentially providing for a new understanding of pesticide and heavy metal implications in AD. Supported in part by NIH R01ES026057.
Numerous epidemiological studies have reported associations between the broad spectrum herbicide paraquat (PQ) and Parkinson’s disease (PD), with findings supported by injection and feeding studies. Despite that inhalation exposure to airborne pesticides, such as PQ, is an occupational and public health concern, the ability of inhaled PQ to reproduce features of PD has not been investigated. The present study was designed to determine if inhalation exposure to PQ would lead to its disposition to the brain, dopaminergic dysregulation, and neurobehavioral outcomes consistent with the trajectory of PD. Adult male and female C57BL/6J mice were exposed to PQ aerosols (130 µg/m³) in a whole-body inhalation chamber for 4hrs/day, 5 days/week for 4 weeks. Subsets of males were sacrificed during and after exposure and PQ concentrations in various brain regions (olfactory bulb, striatum, midbrain, and cerebellum) were quantified via mass spectrometry. Alterations in motor behavior were examined using spontaneous locomotor activity, rotarod, and grip strength. Following the conclusion of behavioral assessment 275 days after the end of exposure, mice were sacrificed and neurotransmitters were measured by mass spectrometry. PQ inhalation resulted in significant concentrations in all examined brain regions, with the highest burden observed in the olfactory bulb (3.34 ± 0.72 ng/g tissue), consistent with normal olfactory translocation. PQ inhalation produced male-specific deficits in locomotor activity and grip strength, but no significant effect on motor coordination on the rotarod apparatus. Critically, PQ inhalation exposure led to a significant male-specific reduction in midbrain dopamine (25%), even 275 days post-exposure. Further, in the striatum, PQ significantly reduced the dopamine metabolite, homovanillic acid (33%), glutamine (21%), serotonin (33%), and its metabolite 5-HIAA (40%), relative to filtered-air controls. These data highlight the importance of inhalation as route of exposure for neurotoxic pesticides in the airborne state and lend biological plausibility to a causal relation between PQ and PD.

Inhalation Dysregulates Neuroimmune Homeostasis and Augments Alzheimer’s Disease-Like Neuropathology

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Increasing evidence associates indoor fungal exposure with deleterious central nervous system (CNS) health, such as cognitive and emotional deficits in children and adults, but the potential impact on CNS disease, particularly Alzheimer’s disease (AD), is poorly understood. To characterize how Aspergillus versicolor, a common opportunistic filamentous fungus species associated with damp environments modifies the CNS transcriptional phenotype, 8-week-old female B6C3F1/N mice were exposed to filtered air, heat-inactivated A. versicolor (3 x 10⁵ spores), or viable A. versicolor (3 x 10⁵ spores) via nose-only inhalation twice a week for 4 weeks. Bulk RNA-seq analysis of the midbrain, the brain region determined to have the largest TNFα neuroinflammation response by RT-qPCR, revealed that 4 weeks of viable A. versicolor exposure resulted in significant changes to transcription of neuroinflammation, glial cell activation, post synaptic, and neurotransmission pathways. To discern the effect of A. versicolor on neurodegenerative disease processes, 8-week-old male 5xFAD mice were exposed to either filtered air or live A. versicolor (3x10⁵ spores) twice a week for 13 weeks. Immunohistochemical analysis of AD-like neuropathology in the cortex demonstrated an increase in the number of Thioflavin S+ plaques in the cortex with exposure, analysis of AD-like neuropathology in the cortex demonstrated an increase in PQ and PD.

The airborne state and lend biological plausibility to a causal relation between exposure. Further, in the striatum, PQ significantly reduced the dopamine mortality and grip strength, but no significant effect on motor coordination on the rotarod apparatus. Critically, PQ inhalation exposure led to a significant male-specific reduction in midbrain dopamine (25%), even 275 days post-exposure. Further, in the striatum, PQ significantly reduced the dopamine metabolite, homovanillic acid (33%), glutamine (21%), serotonin (33%), and its metabolite 5-HIAA (40%), relative to filtered-air controls. These data highlight the importance of inhalation as route of exposure for neurotoxic pesticides in the airborne state and lend biological plausibility to a causal relation between PQ and PD.

5XFAD female mice in the Barnes maze relative to WT. Translocator Protein (TSPO) levels in the 7M 5XFAD female brain relative to WT was inversely associated with NOD-like receptor protein 3 (NLRP3) inflammasome via the Voltage-Gated Protein Channel Hv1

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Paraoquat (PQ) is a widely used herbicide and can increase the risk of developing Parkinson’s disease (PD) by ~2.5 times. PQ treatment of mice induces selective nigrostriatal degeneration, aggregation of α-synuclein, and increased neuroinflammation. Recently, NOD-like receptor protein 3 (NLRP3) inflammasome was increased in the brain of PD patients, indicating a potential role in PD. Hvn1/Hv1 is a voltage-gated proton channel selectively expressed in microglia and other inflammatory cells. It has been shown to be required for NADPH-oxidase (NOX)-dependent production of reactive oxygen species (ROS) under pathological conditions. The purpose of this study was to determine the potential for PQ to prime/activate the NLRP3 inflammasome and the potential for Hv1 to regulate this process. Direct PQ treatment induced Hvn1 mRNA levels 2.3-fold in primary microglia (PMG) and mRNA expression of Nlrp3 in C57. PMG isolated from global Hv1 knockout (Hv1 KO) mice displayed significantly reduced production of ROS and mRNA levels of Nlrp3 and il1β following PQ treatment. PQ treatment of wild-type (WT) PMG increased expression levels of the NLRP3 inflammasome-related proteins including NLRP3, ASC, cleaved caspase-1, and cleaved IL-1β, which were abolished in PMG from Hv1 knockout mice (KO). The ability of PQ to prime the NLRP3 inflammasome was confirmed by increased protein levels of NLRP3, ASC, cleaved caspase-1, and cleaved IL-1β following a second PQ challenge or lipopolysaccharide priming. These effects were attenuated or abolished in Hv1 KO PMG. Similar effects were observed for Il1β measurements in conditioned media. Following a single injection of 10 mg/Kg PQ to C57BL/6J mice, mRNA levels of Hvn1 and IL-1β were increased by 6-fold and 2-fold in the striatum, respectively. As an indicator of NLRP3 activation, ASC protein levels were increased by 6-fold in the striatum and 5-fold in substantia nigra. These effects were abolished in Hv1 KO mouse brain. Collectively, these data demonstrate that direct PQ treatment can both prime and activate the microglial NLRP3 inflammasome and that voltage-gated proton channel Hv1 plays a key role in this process. Supported in part by RO1ES021800 and The Michael J Fox Foundation.

Brain TSPo Levels Are Associated with Sex-Dependent Cognitive Function Deficits in the 5XFAD Animal Model of Alzheimer’s Disease


Alzheimer’s disease (AD) is an irreversible neurodegenerative disease with memory loss and dementia. Neuroinflammation is thought to play an important role in AD pathogenesis and appears to be an early event in the progression of the disease. Here, we used the 5XFAD transgenic mouse model which expresses five of the major human familial mutations associated with AD. These mutations cause this mouse model to develop AD pathogenesis rapidly, with advanced disease observed by 7-10 months of age. We assessed cognitive function using the Barnes Maze in male and female wildtype (WT) and 5XFAD mice at 3 months (3M) and 7 months (7M) of age. We found no significant differences in performance at 3M between WT and 5XFAD male (F1,15=0.583, p=0.457) and female (F1,15=0.438, p=0.518) mice. At 7M, we observed significant differences in learning performance between WT and 5XFAD male mice (F1,15=16.646, p=0.219). However, there was a highly significant difference in the performance of female mice by genotype (F1,15=18.170, p=0.001). That is, we observed a marked impairment in the performance of 5XFAD female mice in the Barnes maze relative to WT. Translocator Protein (TSPo) is a validated biomarker of neuroinflammation that has been used in preclinical animal models of human neurodegenerative disease and in a variety of neurodegenerative conditions including AD. To assess the level of neuroinflammation in the brain of 7M WT and 5XFAD mice, we performed quantitative autoradiography using the TSPo-specific radioligand [3H]-DPA-713. Brain regions analyzed included the cerebral cortex, striatum, septal nuclei, amygdala, hippocampus, thalamus, and hypothalamus. Using an unpaired t-test adjusted for multiple comparisons (p=0.0065), regional analysis of WT and 5XFAD male and female brains at 7M indicated no significant changes in the brain of 5XFAD male mice relative to WT. However, significant increases in TSPo levels were found in the cerebral cortex (p=0.001) and thalamus (p=0.001) of female 7M 5XFAD mice relative to WT. The increase in TSPo levels in the 7M 5XFAD female brain relative to WT was inversely associated with the marked deficits in performance of the 7M 5XFAD female mice.
2663  A Differential Gene Expression Study of Two Siblings with Genetic Risk for Parkinson’s Disease

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Parkinson’s disease (PD) is characterized by the loss of dopaminergic (DAergic) neurons in the substantia nigra. As a complex, multifaceted disorder, accumulating evidence substantiates the role of gene-environment interaction in PD etiology. Previously, we reported a case of two siblings harboring identical compound heterozygous PRKN mutations that exhibited markedly different clinical phenotypes. The older brother displayed symptoms of parkinsonism by his mid-20s, while the younger brother showed exercise-induced dystonia in his mid-30s with no further clinical progression through his late 40s. Furthermore, the two brothers grew up in the same household and neither of them had any unique environmental exposures to chemicals or heavy metals. No family history of neurological diseases or movement disorders have been reported. Hence, we hypothesized that the striking clinicopathological discordance may, in part, be attributed to developmentally relevant expression differences between the two brothers. To address this hypothesis, we set out to investigate the transcriptomic expression profile of iPSC-derived neurons from the brothers. To this aim, we generated four independent iPSC clones from each sibling, differentiated them into DAergic floor plate lineages, and performed RNA-sequencing. The expression profiles were evaluated at days 11 and 25 of differentiation. RNA-sequencing revealed that 34 and 31 genes were significantly differentially expressed between the brothers at days 11 and 25, respectively. Of these, 15 genes that were known to be associated with PD or related environmental risk factors for validation by RT-qPCR. Lineage-specific markers were also examined to ensure proper differentiation. RT-qPCR validated the expression of 14 and 15 genes for days 11 and 25, respectively. Of note, genes involved in glutathione metabolism and cell surface interactions during neurodevelopment displayed the most significant differences between the two brothers. Findings from this study provide insight into the phenotypic discordance between the two brothers. Finally, future studies will advance our understanding of how gene-environment interactions may increase an individual’s susceptibility to, or provide resistance from, the onset of PD.

2664  Perfluorononanoic Acid Impedes Mouse Oocyte Maturation by Inducing Mitochondrial Dysfunction and Oxidative Stress

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Per- and polyfluoroalkyl substances (PFASs), a large group of persistent organic pollutants, are ubiquitous and negatively affect the female reproductive system. Perfluorononanoic acid (PFNA), a member of PFASs, is frequently detected in human blood and tissues. Toxicological studies indicated that PFNA exposure is associated with immunotoxicity, hepatotoxicity, developmental toxicity, and reproductive toxicity in animals. However, there is little information regarding the toxic effects of PFNA on oocyte maturation. In this study, we investigated the toxic effects of PFNA exposure on mouse oocyte maturation in vitro. Our results showed that 600 μM PFNA significantly inhibited germinal vesicle breakdown (GVBD) and polar body extrusion (PBE) in mouse oocytes. Our further study revealed that PFNA induced abnormal spindle assembly, evidenced by malformed spindles and mislocalization of p-ERK1/2 in PFNA-treated oocytes. We also found that PFNA induced abnormal mitochondrial dynamics and increased mitochondrial membrane potential. Consequently, PFNA increased the reactive oxygen species (ROS) level, leading to oxidative stress and DNA damage in oocytes. In addition, the metaphase II (MII) spindle was also disrupted in PFNA-treated oocytes that had completed polar body extrusion after 14 h culture. Collectively, our results indicate that PFNA interferes with oocyte maturation in vitro via disrupting spindle assembly, damaging mitochondria functions, and inducing oxidative stress and DNA damage.

2665  Per- and Polyfluorinated Alkyl Acid Replacement GenX Induces Fibroinflammatory Gene Expression in Human Hepatocytes

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Per and polyfluorinated substances (PFASs) are persistent organic pollutants associated with a myriad of adverse health effects. Their toxicities are dependent on the length of their polyfluorinated tail. Long-chain PFASs have significantly longer half-lives and profound toxic effects compared to their short-chain counterparts. In recent times, production of a short-chain PFAS substitute ammonium, 2,3,3,4,4,4-pentafluorobutyric acid (PFBA) has significantly increased. Nonetheless, however, adverse health effects of GenX are not completely known. In this study, we investigated the adverse effects of GenX on human hepatocytes. Freshly isolated primary human hepatocytes were treated with either 0.1, 10, or 100 μM of GenX for 48 and 96 hours. No cytotoxicity was observed at any dose for each time point. Transcriptomic data generated using microarrays performed on mRNAs isolated from treated and untreated hepatocytes at 96-hour time point were analyzed using Ingenuity Pathway Analysis (IPA) and Gene Ontology (GO). GenX-induced transcriptional changes at 0.1 and 10 μM were more similar to each other than compared to 100 μM treatment. Genes that were significantly altered across all groups were associated with metabolism including lipids, monocarboxylic acid, and ketone metabolism. Changes in gene expression associated with fibrosis and inflammation were more prevalent in the 100 μM treatment group. Correlation analysis of dose and gene expression revealed a total of 576 positively (R > 0.99) and 375 negatively (R < -0.99) correlated genes. Cellular pathways that were affected in a dose-dependent manner were involved in metabolism, inflammation, fibrosis, and proliferation. These data indicate that GenX exposure causes fibroinflammatory and cellular proliferation response in human hepatocytes as a function of dose. These human relevant data will improve our understanding of GenX toxicity and will be beneficial in the risk assessment process.

2666  Dose Additive Developmental Toxicity of a Mixture of Three PFAs (HFPO-DA, NBP2, PFOS) in the Sprague-Dawley Rat

US EPA, Research Triangle Park, NC.

Human biomonitoring data from multiple countries have indicated that virtually all humans carry residues of multiple per- and polyfluorinated alkyl substances (PFASs). Despite the evidence of co-exposure there has been relatively little mixture-based PFASs toxicity research. Studies from EPA have reported that hexafluoropropylene oxide-dimer amide (HFPO-DA, or GenX), Nafion by-product 2 (NBP2), and perfluorooctane sulfonate (PFOS) produce neonatal mortality, among other developmental effects, in rats when orally administered during pregnancy. Here we exposed timed-pregnant Sprague-Dawley rats from gestation day (GD)14-18 (n=6-4 per dose) to investigate maternal and fetal effects and from GD8-postnatal day (PND2) (n=5 per dose) to investigate maternal and postnatal effects. Dams were exposed to fixed-ratio dilutions of an equipotent mixture of HFPO-DA, NBP2, and PFOS where the top dose (100%) contained each chemical at their respective ED50 for neonatal mortality (PFOS, 3mg/kg; NBP2, 10mg/kg; HFPO-DA, 110mg/kg), followed by dilutions of 33, 10, 3, 1 and 0%. Dams exposed GD14-18 displayed increased relative liver weight at ≥33% dose and fetal livers displayed multiple upregulated PPAR target genes at ≥1% dose. GDB-PND2 exposed dams in the 100% dose had reduced weight gain during pregnancy and complete litter loss of all liveborn pups by PND2. Neonatal survival was also reduced on PND2 in the 3 and 33% dose groups and average pup birthweight was reduced at ≥33% dose. Newborn pup serum from the 100% dose had significantly elevated total cholesterol and triglycerides. On PND2, pup body weight was significantly lower and relative liver weight was significantly greater than control in all dose groups. Despite dosing ending at GD14, maternal rats in the 33% dose group had elevated relative liver weight (≥0.99) correlated genes. Cellular pathways that were affected in a dose-dependent manner were involved in metabolism, inflammation, fibrosis, and proliferation. These data indicate that GenX exposure causes fibroinflammatory and cellular proliferation response in human hepatocytes as a function of dose. These human relevant data will improve our understanding of GenX toxicity and will be beneficial in the risk assessment process.
Perfluoroalkyl substances (PFAS) have emerged as global contaminants with wide use in industrial settings, producing non-stick cookware, hydro- and oleophobic textiles, fire-fighting foams, and more. PFAS are ubiquitous, persistent, and mobile in the environment, leading to commonplace exposure. It is estimated that more than 95% of all Americans have detectable serum levels of PFAS. Exposure to PFAS has been linked to cancer, thyroid disease, and immune system dysfunction. Previous research has focused largely on the effects of PFAS on the adaptive immune system, leaving a knowledge gap on what is known about the effects of these compounds on the innate immune system. To bridge this gap, we utilized an in vivo larval zebrafish model and an in vitro human neutrophil-like cell culture model to investigate innate immune function after exposure to environmentally relevant PFAS. First, range-finding studies were performed to determine concentrations at which there was no developmental toxicity or cytotoxicity. Using the established non-teratogenic or non-cytotoxic concentrations, the respiratory burst was measured as a functional readout of innate immune function. In phagocytes, the respiratory burst is the rapid induction of microbiocidal reactive oxygen species in response to infection. Preliminary data revealed that three PFAS inhibited the respiratory burst in vitro. While experiments are ongoing, one of these PFAS also inhibited the respiratory burst in vivo. Potency, as measured by logistic regression, was similar between the two model systems, which may indicate evolutionary conservation between zebrafish and humans in their response to PFAS. Current studies are exploring this hypothesis, whether PFAS-induced immunotoxicity is differentially influenced by chronic or acute exposure, and whether exposure to PFAS can confer susceptibility to infectious disease.

Perfluoroalkyl substances (PFAS) have been widely used in industrial settings due to their use in the manufacture of lucrative non-stick products. However, studies have shown that they are biopersistent and tend to accumulate in the bloodstream over extended periods of time. Furthermore, PFAS are known to demonstrate an overall immunosuppressive effect. Immunosuppression is detrimental to human health because it can increase susceptibility to the adverse respiratory effects of bacteria, viruses and environmental pollution. Perfluoro-2-propoxypropanoic acid (GenX) is classified under the PFAS family and thus is a point of concern for the immune system of individuals exposed. Exposure to GenX may exacerbate lung inflammation through modulating the innate immune response of alveolar macrophages. Lipopolysaccharide (LPS), a component of Gram-negative bacteria, is ubiquitous to the environment and known to induce acute lung inflammation. Thus, GenX may modify the macrophage proinflammatory response induced by LPS to a proliferative response via suppression of NF-kB and activation of the ERK signaling pathway. A recent macrophage cell line (RAW264.7) was stimulated with GenX (5 mM), LPS (1 μg/mL), or both at varying time points (30 min, 1 hour, 24 hours). Cell supernatants were collected after 24 hours to measure IL-6 and CXCL-1 protein levels by ELISA. Cell lysates were collected after 30 minutes and 1 hour to measure αp, ERK and pSTAT3/STAT3 levels by Western Blot. GenX suppressed LPS-induced IL-6 and CXCL-1 expression. Furthermore, GenX and LPS combination suppressed ERK activation level compared to LPS treatment alone. These findings suggest that exposure to GenX could result in increased susceptibility to the adverse respiratory effects of LPS through suppressing the innate immune response of macrophages. Proper immune response. Supported by CHHE Grant P30ES025128.

Perfluoroalkyl substances (PFAS) have been mostly used in industrial settings due to their use in the manufacture of lucrative non-stick products. However, studies have shown that they are biopersistent and tend to accumulate in the bloodstream over extended periods of time. Furthermore, PFAS are known to demonstrate an overall immunosuppressive effect. Immunosuppression is detrimental to human health because it can increase susceptibility to the adverse respiratory effects of bacteria, viruses and environmental pollution. Perfluoro-2-propoxypropanoic acid (GenX) is classified under the PFAS family and thus is a point of concern for the immune system of individuals exposed. Exposure to GenX may exacerbate lung inflammation through modulating the innate immune response of alveolar macrophages. Lipopolysaccharide (LPS), a component of Gram-negative bacteria, is ubiquitous to the environment and known to induce acute lung inflammation. Thus, GenX may modify the macrophage proinflammatory response induced by LPS to a proliferative response via suppression of NF-kB and activation of the ERK signaling pathway. A recent macrophage cell line (RAW264.7) was stimulated with GenX (5 mM), LPS (1 μg/mL), or both at varying time points (30 min, 1 hour, 24 hours). Cell supernatants were collected after 24 hours to measure IL-6 and CXCL-1 protein levels by ELISA. Cell lysates were collected after 30 minutes and 1 hour to measure αp, ERK and pSTAT3/STAT3 levels by Western Blot. GenX suppressed LPS-induced IL-6 and CXCL-1 expression. Furthermore, GenX and LPS combination suppressed ERK activation level compared to LPS treatment alone. These findings suggest that exposure to GenX could result in increased susceptibility to the adverse respiratory effects of LPS through suppressing the innate immune response of macrophages. Proper immune response. Supported by CHHE Grant P30ES025128.

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Viejo, CA; 2Cardno ChemRisk, San Francisco, CA; 3Cardno, Greenback, TN;
Future work will focus on identifying the mechanism behind the observed
locomotor activities in larvae (p<0.05), while PFOA didn't cause any changes
embryonic exposure to PFOS, PFBS, PFBA or GenX induced changes in the
creased mortality of a given chain length. Behavioral analysis showed that
increases with increasing chain length and presence of a sulfonate group in-
PFBS >GenX > PFBA. Based on these results, it is concluded that mortality
parts per billion (ppb; µg/L). The exposure was terminated at 72 hpf, the
test was completed at 120 hpf, and data analyzed with a repeated measures
ANOVA by phase. Results of the 120 hpf-LC50s rank toxicity as PFOS> PFOA>
PFBs >GenX > PFBA. Based on these results, it is concluded that mortality
increased with increased chain length and presence of a sulfonate group in-
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embryonic exposure to PFOS, PFBS, PFBA or GenX induced changes in the
locomotor activities in larvae (p<0.05), while PFOA didn't cause any changes
(p<0.05) indicating different toxicity influences than the mortality results.
Future work will focus on identifying the mechanism behind the observed
behavioral changes.

Per- and polyfluoroalkyl substances (PFAS) are a highly popular class of
synthetic chemicals utilized for their surfactant properties. PFAS are ubiqui-
tously found throughout the environment, including food and water sup-
plies. Epidemiological studies focusing on several model PFAS have found exposure to be associated with chronic diseases such as osteoarthritis and
cardiometabolic disorders. However, little is known regarding the mechanisms underlying the observed toxicities, especially in the context of diets that more appropriately reflect Western-type diets. PFAS exposures are reproducibly associated with increased morbidity and mortality (e.g., cancer), while animal studies conversely report primarily hypocholesterolemia. To study the cardiometabolic toxicity of PFAS exposure, we have characterized a novel mouse model that results in increased circulating cholesterol levels in both male and female mice. C57Bl/6 mice were fed a 0.15% cholesterol diet and ex-
posed to water containing a mixture of 5 common PFAS (PFOS, PFBS, PFNA, 
PFHxS, and GenX) for 12 weeks. PFAS exposure via drinking water resulted in
increased hepatic steatosis, demonstrated by 3-fold increased liver weight, increased hepatic inflammation, as well as increased hepatocyte apoptosis and necrosis. Metabolomics analysis revealed PFAS exposure modulated multiple metabolic pathways, including lipids, bile acids, and fatty acid
β-oxidation. However, measured PFAS levels and exposure effects were not
necessarily the same between genders. In fact, plasma PFOA concentrations in
PFAS-exposed females were found to be nearly 2-fold higher than males, while PFOS concentrations were significantly lower (0.77-fold reduced). Also,
plasma cholesterol levels were elevated to differing degrees in both male
and female mice due to PFAS exposure (1.33- and 1.62-fold increase, respec-
U.S. EPA, Research Triangle Park, NC; 2US EPA/CCTE, Cincinnati, OH; and 3US EPA/CCTE,
Research Triangle Park, NC.

A vast amount of chemical toxicology and property data is publicly accessi-
ble via the Internet. However, these data are often uncurated, unreferenced, and distributed across many data sources. Additionally, despite the prolif-
eration of data, certain classes of chemical remain poorly characterized ex-
perimentally, notably including per- and polyfluoroalkyl substances (PFAS).
This project seeks to develop a systematic architecture to consolidate existing
chemical data for use in quantitative structure-activity relationship (QSAR)
modeling. Such a process will both increase the quality and quantity of data

2672 Exposure to a Mixture of Legacy and
Emerging Per- and Polyfluoroalkyl Substances (PFAS) Modulates Lipid
Metabolism in Mice
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Detroit, MI.

Per- and polyfluoroalkyl substances (PFAS) are ubiquitous in the environ-
ment. PFAS do not readily degrade in the environment, may bioaccumulate through
the food web, and have the potential to cause adverse effects to the health of humans and wildlife. Unlike other persistent chemicals, these chemicals are highly soluble in water and tend to partition to surface and
groundwater rather than soil and sediment in the environment. The current
study explores a modeling approach that could be used to evaluate human
exposure to newer and less studied PFAS. This study focuses on perfluorcoc-
tanic acid (PFOA) and perfluorooctane sulfonic acid (PFOS), since these are the
two most commonly studied PFAS, facilitating validation of the approach.
Specifically, the Exposure and Fate Assessment Screening Tool (E-FAST) was
used to estimate exposure to PFOA and PFOS via ingestion of drinking water and
consumption of fish following a discharge from six wastewater treatment
facilities (3 facilities discharged into rivers and 3 facilities discharged
into ocean), using a wide range of hypothetical industrial release scenarios
(0.001-10 kg PFOA or PFOS per site per day). Our preliminary results for adult
exposure to the potential lifetime average daily dose (LADDexp) values for consumption of PFOA-containing fish ranged from 4.30x10^4 to
4.86x10^4 μg/kg/day for freshwater bodies and from 8.78x10^-7 to 2.77x10^-7 μg/kg/day for marine water bodies. The LADDexp values for consumption of PFOS-containing fish were from 1.32x10^-4 to 1.44x10^-4 μg/kg/day for fresh-
water bodies and from 2.71x10^-6 to 8.52x10^-7 μg/kg/day. The LADDexp values for ingestion of PFOA- and PFOS-containing drinking water ranged 1.59x10^-12 -2.04x10^-16 and 1.07x10^-12 -2.04x10^-16 mg/kg/day, respectively. We conclude that the outlined approach will serve as a useful tool to predict the human ex-
posure to newer and less studied PFAS and potentially other chemicals with
limited information. Validation of this approach by conducting literature review will also be described.

2673 Using E-FAST Platform to Predict Human
Exposure to PFAS
M. T. Donnell1, A. Massarsky1, E. de Gandiaga1, J. S. Koza1, L. C. Garnick2, 
S. M. Bartell2, J. A. Kubitz3, and A. D. Monnot2. 
1Cardno ChemRisk, Aliso Viejo, CA; 2Cardno ChemRisk, San Francisco, CA; 
3Cardno, Greenback, TN; and 4Cardno, Crystal Lake, IL.

Per- and polyfluoroalkyl substances (PFAS) are ubiquitous in the environ-
ment. PFAS do not readily degrade in the environment, may bioaccumulate through
the food web, and have the potential to cause adverse effects to the health of humans and wildlife. Unlike other persistent chemicals, these chemicals are highly soluble in water and tend to partition to surface and groundwater rather than soil and sediment in the environment. The current study explores a modeling approach that could be used to evaluate ecotoxicity of the newer and less studied PFAS, thereby reducing the need for animal testing. This study focuses on perfluorooctane sulfonic acid (PFOA) and perfluorooctane sulfonic acid (PFOS), since these are the two most commonly studied PFAS, facilitating validation of the proposed approach. In the first step, the Ecological Structural Activity Relationships (ECOSAR) was used to derive acute and chronic toxicity threshold values for PFOA and PFOS for several aquatic species, including fish, Daphnia, mysid, and green algae. In the second step, the Exposure and Fate Assessment Screening Tool (E-FAST) was used to derive the surface water concentrations for PFOA and PFOS following a discharge from six wastewa-
ter treatment facilities (3 facilities discharged into rivers and 3 facilities dis-
charged into ocean), using a wide range of hypothetical industrial release scenarios (0.001-10 kg PFOA or PFOS per site per day). In the third step, risk as-
4Cardno, Crystal Lake, IL.

2674 Using ECOSAR and E-FAST Platforms to 
Predict Ecological Risks of PFAS
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S. M. Bartell2, J. A. Kubitz3, and A. D. Monnot2. 
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Systematic Data Gathering for PFAS Toxicity and Property Modeling
G. Sinclair1, C. Ramsland1, T. Martin2, and A. Williams3. 
1US EPA, Research Triangle Park, NC; 2US EPA/CCTE, Cincinnati, OH; and 3US EPA/CCTE, 
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A vast amount of chemical toxicology and property data is publicly accessi-
ble via the Internet. However, these data are often uncurated, unreferenced, and distributed across many data sources. Additionally, despite the prolif-
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2675 Systematic Data Gathering for PFAS Toxicity
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available to model the toxicology and properties of PFAS, as well as permitting identification of present gaps in data collection. Thirteen chemical data sources were selected, including academic (e.g. PUBChem), governmental (e.g. ECHA), and commercial (e.g. LookChem) vendors. In addition, three sets of literature data were manually compiled for inclusion. Initially, all data were collected and stored in their original format. Using tailored processing tools developed in Java for the project, these data were translated to a structured intermediate JSON format retaining all original information. They were then standardized to a final JSON format which highlights the specific properties of interest for QSAR modeling and normalizes quantities including units, measurement methods, and remarks for each property. These 199,000+ standardized data points were subsequently integrated into a single SQL database. The unique combination of chemical identifiers for each data point can be mapped to a substance ID in the EPA’s Distributed Structure-Searchable Toxicology Database, allowing that data to be associated to a QSAR-ready SMILES string in order to retrieve molecular descriptors for model development. This project further investigates understanding of the gaps in available property data. Of particular concern is the known problem of modeled data not disclosed as such, and thus improperly included in sources of experimental data; for instance, in the EPA’s High Production Volume Information System, only ~230 entries are appropriately labeled as estimated, but up to ~2,000 more include indications in remarks that the results may be predicted rather than experimentally determined. The consolidated data, curated procedurally for this and other problems, lays the groundwork for a broad effort to modeling the toxicological and physicochemical properties of PFAS, and the architecture and tools developed in support of it will permit ongoing expansion as new data continue to become available.

**2676** Systematic Evaluation of the Developmental Toxicity of Per- and Polyfluoroalkyl Substances (PFAS) in Zebrafish

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Thousands of per- and polyfluoroalkyl substances (PFAS) are on the global market, and many of these are ubiquitously detected in the environment and humans. Some PFAS have been shown to induce toxic effects, however, most studies have focused on just a handful of compounds with predominately carboxylic acid and sulfonic acid functional head groups. Given the large number of PFAS and their structural diversity, there is a need for additional toxicological investigation into PFAS with additional functional head groups. In this study, we leveraged zebrafish as a model to evaluate the developmental toxicity of a library of 23 PFAS from 4 functional head group categories: carboxylic acids (PFCCs, 10), sulfonic acids (PFSSs, 5), sulfonamides (FASAs, 5), and fluorotelomer sulfonic acids (FTSS, 3). Developmental toxicity assays (n = 36) were performed by statically exposing dechorionated zebrafish embryos to 8 concentrations of PFAS using methanol as a carrier solvent, ranging from 1-100 µM, in 96-well plates from 6 to 120 hours post fertilization (hpf). Embryonic photomotor response (EPR) behavior was assessed at 24 hpf, followed by 10 morphological endpoints and larval photomotor (LPMR) response at 120 hpf. All morphological effects were combined to generate concentration-response curves, from which we derived EC50 values. Our results indicate that PFAS toxicity follows the trend FASAs > PFCCs > PFSSs = FTSS. Among the FASAs, EC50 decreased with increasing fluorinated chain length; perfluorooctanoic acid (PFOA) elicited an EC50 of 14.6 µM, and perfluorooctane sulfonamide (FOSA) an EC50 of 3.27 µM. In the PFSA category, perfluorooctane sulfonic acid (PFOS) induced an EC50 of 10.7 µM, with additional results pending. Conversely, PFCCs and FTSS did not cause significant developmental toxicity. Nearly all 23 PFAS (regardless of head group) induced abnormal LPR, but interestingly, only FASAs elicited aberrant EPR. Using these results, we have prioritized individual PFAS for further evaluation. We are conducting transcriptomic investigations of PFAS homologous series in 48 hpf zebrafish, anchoring concentrations to observed morphological effects in the FASA and/or PFSA groups. In doing so, we will further elucidate PFAS modes of action and structure-bioactivity relationships.

**2677** Predicting the Effect of PFAS Mixtures on Nuclear Receptor Activity

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Per- and polyfluoroalkyl substances (PFAS) are a group of more than 4,000 structurally related synthetic chemicals. Human exposure to PFAS is ubiquitous, with multiple PFAS detected in food, drinking water, and household dust. Evidence from animal toxicity studies and human epidemiology suggests that different PFAS may operate through shared mechanisms such as the activation of nuclear receptors like peroxisome proliferator activated receptor α (PPARα) and constitutive androstane receptor. Nuclear receptor modulation is a likely molecular initiating event underlying the sensitive metabolic effects associated with exposure to multiple different PFAS. In this analysis, we first review existing approaches to modeling PFAS mixtures. Concentration addition has been used to model the effects of PFAS mixtures on the activation of PPARα using GAL-4 driven reporter assays and cytotoxicity with human-like hepatoma cells. The effects of PFAS mixtures have been modeled in vivo with relative potency factors and toxic equivalences using liver hypertrophy as an endpoint. Second, we introduce Generalized Concentration Addition (GCA) as an approach to model the biological effects of PFAS mixtures. We generated individual dose-response data for full and partial PPARα agonists (pemflubate, GW7647 and ETYA) and a PPARα antagonist (GW6471) using the Cos7 cell line transfected with full length human PPARα and a peroxisome proliferator response element-driven luciferase reporter. We then used the reporter assay to assess PPARα activation by mixtures of the compounds using a novel design. Data for individual compounds fit the PPARα dose-response function well. Application of GCA produced accurate predictions for PPARα activation by mixtures of two compounds. We then examined the efficacy of GCA in modeling the effects of PFAS on PPARα-driven reporter gene expression, including PFOS, PFOD, and GenX. GCA also accurately predicts activation of PPARα by complex PFAS mixtures. Currently, we are investigating the ability of GCA to predict the effect of PFAS mixtures on endogenous target gene expression (PDK4) in a human hepatocyte model (HepaRG cells).

**2678** PFOA Modifies Fatty Acid and Triglyceride Homeostasis in a Humanized PPARα Mouse Model Fed an American Diet

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Per- and polyfluoroalkyl substances are pervasive in the environment because of their persistence and extensive use in fluoropolymer production, consumer products and aqueous film forming foams used in firefighting. Daily exposures occur via PFAS contaminated food, drinking water, dust and air, resulting in nearly universal detection in people examined. Dyslipidemia (elevated triglycerides, decreased high density lipoprotein cholesterol) and increased serum LDL-C are major contributors to cardiovascular disease. Despite evidence that PFAS induce dyslipidemia, these effects of PFAS have received little toxicological research focus, largely because there is no suitable animal model. Previously, we showed that perfluorooctanoic acid (PFOA) exposure in mice expressing human peroxisome proliferator activated receptor α (PPARα) fed a diet based on the “What we eat in America” induced PPARα and constitutive androstane receptor target gene expression in liver, dysregulated expression of genes involved in cholesterol homeostasis and increased serum LDL-C. Here, we tested the hypothesis that PFOA dysregulates liver and serum lipids in the same mouse model. PFOA exposure in drinking water (3.5 mg/l) for 6 weeks resulted in a serum PFOA concentration of 48 ± 9 μg/ml, a serum level found in highly exposed fluoropolymer workers. PFOA exposure had a significant impact on the liver lipidome, increasing lipid content while differentially modifying concentrations of specific lipid classes (e.g. increasing triglycerides, decreasing sphingomyelins). PFOA also modified fatty acid and triglyceride homeostasis-related gene expression in liver, in at least a hPPARα-dependent manner. PFOA modestly increase serum triglycerides in female hPPARα mice and robustly increased serum triglycerides in PPARα null mice. PFOA induced little effect on serum triglycerides in male mice of either genotype. A literature-based analysis of effects of PFOA on serum triglyceride and cholesterol across multiple mouse models revealed a non-monotonic dose response. PFOA clearly has a significant effect on liver and serum lipids in a human relevant model at human relevant body burdens. More research is needed to test effects of lower body burdens at steady state, more like exposed communities, which we hypothesize will increase both serum cholesterol and triglycerides.
Perfluorooctane sulfonic acid (PFOS) has been used as a fluorosurfactant in flame retardants, non-stick coatings, and textiles since the 1940s resulting in exposure, especially in regards to the respiratory system are poorly understood. Although PFOS is ubiquitously detected in human serum, umbilical cord blood, and breast milk, yet the adverse health effects of PFOS exposure resulted from inhalation exposure are not clearly understood. PFOS is a major risk factor for asthma which affects over 300 million people worldwide. PFOS is known to disturb the epithelial barrier and induce inflammation in the respiratory system. However, the exact molecular mechanism by which PFOS exerts its toxic effect is not fully understood. Recent studies have suggested that PFOS exposure to bronchial epithelial cells (16HBE) would disrupt barrier function and decrease transepithelial electrical resistance (TEER) and increase in FITC dextran permeability at 15μM at 72 hours. Cell viability was not affected by these treatments as shown by LDH release assay in the conditioned media. These changes were accompanied by a significant decrease in the protein abundances of claudin-4, occludin, and zonula occludens-1 (ZO-1) and increased immunofluorescence. In this study, we sought to better understand the potential effects of old- and new-generation PFASs on human embryonic development. We induced murine embryonic stem cells to differentiate into the epithelial cell lineage and exposed them to PFOS or PFOA at various concentrations. Both PFOS and PFOA elicited a strong effect on cardiomyocyte differentiation at non-cytotoxic concentrations, with PFOS being more potent than PFOA. Both compounds also reduced the EB size at the highest tested concentrations. GenX did not initially affect differentiation in the first cell line tested, but induced a weak dose-dependent effect when tested in a second hiPS cell line. Gene expression analysis of the cardiomyocytes at experiment termination showed that PFOS increased expression of the early cardiac marker Isl1, while PFOA decreased expression of the cardiomyocyte marker Mvh7. This suggests that PFOS, PFOA, and potentially GenX disturb cardiomyocyte differentiation. However, GenX did not affect expression of any of the analyzed genes. Our study shows that PFOS, PFOA, and GenX have the potential to disrupt the early development of the embryo.

**2680 The Role of Human and Mouse PPARα in Modulating the Hepatic Effects of PFOS in Mice**

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Perfluorooctane sulfonic acid (PFOS) is an exceptionally stable environmental contaminant found in tissues of many species. PFOS can activate peroxisome proliferator-activated receptor alpha (PPARα) and activation of this receptor can increase hepatic peroxidation in rodents. In the present work, the role of PPARα in mediating the hepatic effects of PFOS was examined in short-term studies using male wild type, Ppara-null and humanized PPARα mice. Administration of dietary PFOS (0.003% or 0.006%) caused an increase in hepatic concentrations of PFOS in liver and serum of mice that modeled PFOS exposure in humans. Expression of the PPARα target gene, Cyp4a10, was significantly increased in wild type mice when compared to the null or Ppara-null mice. Expression of the constitutive androstane receptor (CAR) target gene, Cyp2b10, and the pregnane X receptor (PXR) target gene, Cyp3a11, was higher in response to PFOA administration in all three genotypes compared to controls. Interestingly, relative liver weight was higher following exposure to dietary PFOS in all three genotypes as compared to the control groups. Histopathological examination of liver sections revealed hepatoellular hypertrophy in all three genotypes in response to dietary PFOS. However, the incidence of hepatoellular hypertrophy was lower in Ppara-null mice (3/6) than wild type (6/6) and Ppara (6/6) mice. These results indicate that mouse PPARα can be activated by low dose exposure to PFOS and this effect can be mediated by both the mouse and human PPARα. The liver hypertrophy caused by PFOS appears to require the mouse PPARα at least in part as this phenotype was mitigated in part in Ppara-null mice compared to wild-type and Ppara mice. Given the changes in expression of genes regulated by other transcription factors caused by PFOS exposure, PFOS-induced hepatic effects are also likely influenced by CAR and/or PXR. Collectively, these results provide support for further studies to distinguish between the effects mediated by more than one receptor in response to exposure to PFOS. Supported by an unrestricted gift from 3M Company.

**2681 Alterations in Lung Epithelial Barrier Integrity May Be Associated with Downregulation of AKT Signaling and Tight Junction Proteins by Perfluorooctane Sulfonic Acid (PFOS)**


Perfluorooctane sulfonic acid (PFOS) has been used as a fluorosurfactant in flame retardants, non-stick coatings, and textiles since the 1940s resulting in widespread pollution of both environmental and biological systems. Recent epidemiological studies indicate PFOS may promote the development of asthma, a chronic inflammatory respiratory disease that affects over 300 million people worldwide. PFOS is ubiquitously detected in human serum, umbilical cord blood, and breast milk; yet, the adverse health effects of PFOS exposure, especially with regards to the respiratory system are poorly understood. A major risk factor for asthma is a defective lung epithelial barrier which may facilitate allergen processing and immune cell activation. Although PFOS causes barrier dysfunction in models of the lung-blood-testis barriers, less is known about its effects on the lung epithelial barrier. We hypothesized that PFOS exposure to bronchial epithelial cells (16HBE) would compromise barrier integrity and tight junction function. 16HBE cells were exposed to various doses (1-25μM) of PFOS in transwell cultures for barrier function assays or standard cell culture plates for protein analysis. Bilateral PFOS exposure resulted in a dose-dependent decrease in transepithelial electrical resistance (TEER) and an increase in FITC dextran permeability at 15μM at 72 hours. Cell viability was not affected by these treatments as shown by LDH release assay in the conditioned media. These changes were accompanied by a significant decrease in the protein abundances of claudin-4, occludin, and zonula occludens-1 (ZO-1) and increased immunofluorescence. In this study, we sought to better understand the potential effects of old- and new-generation PFASs on human embryonic development. We induced murine embryonic stem cells to differentiate into the epithelial cell lineage and exposed them to PFOS or PFOA at various concentrations. Both PFOS and PFOA elicited a strong effect on cardiomyocyte differentiation at non-cytotoxic concentrations, with PFOS being more potent than PFOA. Both compounds also reduced the EB size at the highest tested concentrations. GenX did not initially affect differentiation in the first cell line tested, but induced a weak dose-dependent effect when tested in a second hiPS cell line. Gene expression analysis of the cardiomyocytes at experiment termination showed that PFOS increased expression of the early cardiac marker Isl1, while PFOA decreased expression of the cardiomyocyte marker Mvh7. This suggests that PFOS, PFOA, and potentially GenX disturb cardiomyocyte differentiation. However, GenX did not affect expression of any of the analyzed genes. Our study shows that PFOS, PFOA, and GenX have the potential to disrupt the early development of the embryo.

**2682 Investigating the Effects of Perfluorooctanoic Sulfonate (PFOS) and Ethanol on Fatty Liver Disease Using a Modified NIAAA Model**

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Poly- and Perfluoroalkyl Substances (PFAS) are a large, synthetic family of surfactant-like compounds that are considered persistent organic pollutants. PFAS exposure in humans is associated with several adverse health effects including fatty liver disease (FLD). While mechanistic studies on PFAS toxicity are still emerging, one of the most consistent links to altered lipid homeostasis caused by PFAS is activation of the peroxisome proliferator activating receptor alpha (PPARα). Apart from environmental pollutants, factors such as excessive alcohol consumption can also contribute to FLD. However, to date, there are no known reports on the impact of PFASs and ethanol co-exposures in liver injury. The current pilot study aims to characterize the effects of exposure to Perfluorooctanoic Sulfonate (PFOS), a prominent PFAS, in mice consuming an alcohol diet. Male C57BL/6 mice were fed ad libitum Lieber-DeCarli diet with 5% ethanol or pair-fed (0% ethanol) for 15 days. Beginning on day 6, mice were exposed to 1 mg/kg/day PFOS or vehicle (2% Tween-80) via oral gavage. At euthanasia, blood and tissue samples were collected for downstream analyses. PFOS activated hepatic PPARα evident by target gene Cyp4a10 induction. Steatosis was induced by increased liver-to-body weight ratio and histological (H&E) staining in the ethanol+PFOS group. Ethanol consumption resulted in increased hepatic and serum triglycerides but decreased hepatic cholesterol levels. In terms of liver injury and inflammation, elevated liver enzymes (ALT and AST), typically seen in chronic binge ethanol model, were observed. Additionally, hepatic IL-6 expression was significantly increased in ethanol+PFOS group; however, serum IL-6 was increased with ethanol consumption only. Hepatic expression of genes involved in fatty acid synthesis were primarily decreased due to PFOS or ethanol exposure; however, fatty acid transporters were differentially affected by PFOS or ethanol exposure. Overall, this pilot study indicated a possible interaction between ethanol consumption and PFOS exposure. Ethanol showed predominant effects on liver injury endpoints whereas PFOS exposure differentially impacted lipid metabolism endpoints. Future studies will include further developing this ethanol+PFOS model to adapt to other toxicant exposures and investigating mechanisms of ethanol+toxicant interactions in FLD.

**2683 A Quantitative Structure-Activity Relationship (QSAR) Model to Estimate Half-Lives of Perfluoro-alkyl Substances (PFAS) in Multiple Species**

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Perfluoroalkyl substances (PFAS) are a diverse group of synthetic chemicals commonly found in body tissues. Modeling their toxicokinetics is an important part of risk prioritization and assessment. However, in vivo assays of PFAS have demonstrated that half-life (t1/2) in serum, a key toxicokinetic parameter, can vary considerably among species, by different innate species for a given chemical. This makes read-across methods of predicting t1/2 for non-assayed PFAS chemicals unreliable. In this study, we use a machine learning approach to predict serum half-life in multiple species.
approach to construct Quantitative Structure Activity Relationship (QSAR) models of in vivo $t_{1/2}$ for PFAS chemicals. From a curated set of literature, we assembled a training set of in vivo serum $t_{1/2}$ values (89 data points) for 11 PFAS chemicals across 4 species (human, monkey, rat, mouse). Concurrently, we assembled a diverse predictor set (29) that included chemical structures, physiochemical descriptors and kidney characteristics. From this we built a random forest model among the training set (10x other acid, 10 xperfluoroalkyl sulfonate), using $t_{1/2}$ data points binned into very fast ($\leq 1$ day), fast ($>1$ day and $\leq 1$ week), slow ($>1$ week and $\leq 2$ months), and very slow ($>2$ months) categories. The model had an average accuracy of $84.3 \pm 0.012$% SE. Lipophilicity (LogP) and High-Performance Liquid Chromatography (HPLC) retention time were ranked as the most important parameters. When the model is applied to 6648 chemicals of the DStox PFAS list for humans, approximately 90% are predicted to fall into the very slow bin, with the remainder falling into the slow and fast bin. This work represents a first pass, and independent data to validate model predictions is currently limited. Overall, however, our initial results indicate that many PFAS chemicals would be expected to persist for long periods of time (months to years) within human tissues. The views expressed here are those of the authors and do not necessarily represent the views or the policies of the US Environmental Protection Agency.

2684 Transcriptional Response to Combined Binary Exposures to PFAS in FaO Cells

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Per- and polyfluoroalkyl substances (PFAS) represent a large class of structurally diverse chemicals of increasing public concern, mostly due to their chemical stability and undetected toxicity profiles. Adverse effects implicated for PFAS include liver damage and the associated metabolic dysregulation, immune and thyroid toxicity, reproductive toxicity, and some cancers. The broad commercialization and environmental distribution of PFAS has drawn attention to the need for understanding risks associated with combined exposure to multiple PFAS in complex mixtures. The purpose of this investigation was to assess the response to binary combinations of PFAS elicit a dose or response additive effect. Exposure of FaO rat hepatoma cells for 24 hr to 25μM-200μM of the 4- and 8-carbon perfluorocarboxylic acids (PFOA and PFOA) or the 4, 6, and 8-carbon perfluorobutanesulfonic acids (PFBS, PFHxS, and PFOS, respectively) individually caused a dose-dependent increase in PPARα-regulated peroxisomal bifunctional enzyme (Ehhadh) gene expression. In each case potency increased with carbon number, with the carboxylates elicits a greater transcriptional response than the corresponding sulfonate. Combined exposure to PFOA and PFBA produced a response that was significantly less than either dose or response additive. Combinations of PFOA and PFOS yielded a response additive effect that was significantly less than that predicted by PFOA bioequivalence. Finally, combined exposures to low concentrations of PFOS and either PFBS or PFHxS produced a transcriptional response that was additive but significantly less than the sum of the individual responses. The results of this investigation demonstrate a lack of bioequivalence among the five structurally related PFAS, and that the transcriptional response to combined exposures is consistently at or below that predicted by either dose or response additive. This research was supported in-part by the 3M Co. (kbw) and a Dean’s Research Scholarship in memory of Dr. Arthur G. Johnson (jok).

2685 Effects of a Perfluoroether Acid, an Understudied PFAS, on Developing Mice

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Perfluoro-2-methoxyacetic acid (PFMOAA) was the most prevalent per- and polyfluoroalkyl substance (PFAS) recently detected in the Cape Fear River of North Carolina. It is a short-chain perfluoroether acid (PFEA), a byproduct of fluorochemical manufacturing and is used to understand with respect to its toxicity. Well-studied PFAS are known to affect the adult and developing immune systems. Perturbations in immune development are known to play a role in immune system dysfunction, influencing the susceptibility to many immune-related conditions across a lifetime. As part of an effort to evaluate immunotoxicity of PFAS in adult and developing organisms, we investigated developmental effects of PFMOAA in mice. We hypothesized that gestational exposure to PFMOAA would impact developmental endpoints known to be affected by PFAS. Adult female C57BL/6 mice were paired with adult C3H mice for gestation day (GD) 1 - 17. On postnatal day (PND) 1, litters were weighed, sexed, and culled to 3 males and 3 females/dam. Dams that did not produce litters were euthanized a few days after complection of dosing. Uterus, ovaries, and livers were removed, weighed, and serum was collected and stored for future analysis. Liver weight increases are a biomarker for PFAS exposure in rodents and a measure of relative potency when comparing PFAS. Liver weights for the non-pregnant dams exposed to PFOS increased by 65% relative to weights of livers from control dams. Liver weights of non-pregnant dams exposed to PFMOAA did not change. Wet weights of uterine and placental tissues of dams are described. PFMOAA or PFOS. Oral exposure to PFMOAA did not produce differences in percent weight gain in dams or to the sex ratio of pups/litter. Exposure to PFMOAA did not appear to impact average litter sizes or pup weights at PND1; PFOS exposure did appear to decrease pup weights by about 10% relative to pups exposed to vehicle alone, and note that PFMOAA did not produce detectable changes to developmental biomarkers; additional work is underway to determine impacts of this developmental exposure on the immune system. As PFMOAA was one of the most abundant PFEAs detected in the Cape Fear River, additional data about its potential toxicity is critical to ensure appropriate health protection for those who have consumed it in their drinking water.

2686 Key Methodological Considerations for Dermal Permeability Studies of Per- and Polyfluoroalkyl Substances (PFAS)

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Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are found in some consumer products due to their heat resistance and durability. However, there is potential for these substances to bio-accumulate in humans. It is relevant to investigate biological effects of these chemicals, as studies have suggested early life exposure may impact human developmental outcomes specifically within the first year of life. The objective of the current study was to determine if a relationship exists between detectable levels of certain PFAS and anthropometric measurements in adolescents ages 12-18. The three PFAS examined were: Perfluorodecanoic acid (PFDeA), 2-(N-methylperfluorooctanesulfonamido)acetic acid (Me-PFOSa-AcOH), and Perfluoroundecanoic acid (PFUa). The data was obtained from the 2015-2016 National Health and Nutrition Examination Survey (NHANES), and concentrations were classified as above or below the lower limit of detection (LLOD) of 0.10 ng/mL for each subject (n=311). T-tests were performed on the geometric mean (GM) to assess differences in weight, waist circumference, BMI, and height between those above and below the LLOD. Stratification held the effects of height and age constant. Overall, there were significant associations with PFUA and waist circumference (GMnondetect = 77.69 cm, GMdetect = 82.56 cm, p=0.004), weight (GMnondetect = 61.19 kg, GMdetect = 65.58 kg, p=0.041), and BMI (GMnondetect = 22.47 kg/m², GMdetect = 24.24 kg/m², p=0.027). When stratifying by gender, only males showed an association between PFUA and waist circumference (GMnondetect = 75.76 cm, GMdetect = 82.57 cm, p=0.003) and BMI (GMnondetect = 21.59 kg/m², GMdetect = 23.94 kg/m², p=0.120). Unadjusted for gender, a relationship between decreased height and PFUA concentrations above the LLOD was demonstrated only in age 14, and the relationship between PFDeA and reduced waist circumference was significant only in age 18. No associations were found with Me-PFOSa-AcOH and anthropometric parameters. The mean of each body measurement was significantly lower in the PFUA detect group than in the non-detect group for the total sample. This suggests that PFUA exposure levels above the LLOD in adolescents may be related to smaller waist circumference, weight, and BMI. This effect was significant in males but not females.
ies used acetone as the vehicle for PFOA. We conclude that these studies should be interpreted with caution as acetone enhances dermal penetration of amphiphatic compounds, but these studies may be useful as representing a potential worst-case scenario in occupational settings that involve frequent dermal exposure to acetone (e.g., nail salon workers). Two studies measured in vitro dermal permeability coefficients (Kp) of PFOA. Methodological attributes shared by most of these studies that are appropriate for future dermal uptake studies of PFAS are 1) use of human epidermal membranes as a model for human skin; 2) use of non-acetone solvents (i.e., water or citrate buffer) as the vehicle; and 3) measurement of Kp at pH = 5.01-5.5 to ensure that PFAS were fully ionized. We note that the measured Kp values of PFOA differed between the two studies by two orders of magnitude likely due to differences in the tested concentrations or viscosity of the test materials. However, both Kp values were two to four orders of magnitude below those of compounds considered to have high dermal penetration (e.g., benzene or nicotine), indicating that PFOA demonstrates little dermal penetration under environmentally relevant conditions. We conclude that dermal uptake of PFAS remains an important data gap, but that the available literature indicates dermal absorption is unlikely to be a significant exposure pathway for PFOA and PFAS with similar chemical properties.

2688 Reduction of the Bioavailability of PFAS (Per- and Polyfluoralkyl Substances) from Soil and Their Translocation to Plants in the Presence of Parent and Processed Montmorillonite Clays
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Consumption of food and water contaminated with per- and polyfluoralkyl substances (PFAS) is a significant risk for human health; thus, limited data on high affinity sorbents that can be used to reduce the bioavailability of PFAS from soil and translocation to plants and garden produce. To address this need, montmorillonite clay was amended with the nutrients necessary to denote a hydrophobicity of the sorbent and the interlayer space. In this study, the binding of parent and ameliorated clays to PFOA (perfluorooctanoate) and PFOS (perfluorooctanesulfonate) was characterized. Isothermal analyses were conducted at pH 7 and ambient temperature to simulate environmentally relevant conditions. The data for all tested sorbents fit the Langmuir model indicating saturable binding sites with high capacities and affinities under neutral conditions. Amended montmorillonite clays had increased capacities for PFOA (0.51 and 0.61 mol kg⁻¹) compared to the parent clay (0.37 mol kg⁻¹). Molecular dynamics (MD) simulations suggested that hydrophobic and electrostatic interactions at the terminal fluorinated carbon chains of PFAS compounds were major modes of surface interaction. The safety and efficacy of the clays were confirmed in a living organism (Hydra vulgaris), where clays (at 0.02% inclusion) significantly reduced PFOA and PFOS toxicity (p < 0.01). Importantly, soil studies showed that 2% sorbent inclusion could significantly reduce PFAS bioaccessibility from soil (up to 60%). Studies in plants demonstrated that inclusion of 2% sorbent significantly reduced PFAS residues in cucumber plants (p ≤ 0.05). These results (up to 60%) suggest that nutrient-amended clays could be included in soil to decrease PFAS bioavailability and translocation to plants.

2689 Effect of Subacute Exposure to PFOS and GenX on Gut Microbiota-Host Metabolome Homeostasis
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The disturbance of the normal gut microbiota is a well-known consequence of chemical toxicity and can lead to various adverse health effects in humans. Similarly, the gut microbiota can interact with the toxic environmental chemicals in the gut and modulate their mechanisms of absorption, metabolism, disposition, and excretion. Considering the high level of contamination of our environment with Perfluoroalkyl and polyfluoroalkyl substances (PFAS), this study was conducted to determine the key changes induced by PFAS exposure on the gut microbiota. Their Translocation to Plants in the Presence of Parent and Processed Montmorillonite Clays. Studies in plants demonstrated that inclusion of 2% sorbent inclusion could significantly reduce PFAS bioaccessibility from soil (up to 60%). Currently, collectively, our results suggest that PFOS and GenX exposure lead to key alterations in the gut microbiota differentially in the small intestine and colon, and significant alterations in liver metabolome; contributing to microbiome toxicity, hepatotoxicity, and metabolic disorders.

2690 Comparing an Acceptable Exposure Level Based on In Vitro Studies of PFOA Hepatotoxicity to Levels Measured in Epidemiologic Studies
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Acceptable exposure levels are generally calculated based on animal experiments. It has been shown that data generated using animal models may not adequately predict adverse outcomes in humans. In vitro tests using human cells combined with biological modeling may offer a viable alternative. We conducted a case study to derive acceptable exposure levels for PFOA based on results from in vitro hepatotoxicity assays, and compared these exposure levels with those measured in epidemiological studies reporting associations between PFOA and hepatotoxicity. Points of departure (PODs) were determined based on no observed adverse effect levels (NOAELs) from in vitro studies of PFOA hepatotoxicity in human cells. Because the concentration of PFOA in medium containing albumin stays similar to the initial medium concentration over time, and because PFOS is highly bound to albumin, we assumed that concentrations in vitro systems including albumin can be translated into plasma concentrations in humans. Daily intakes associated with these plasma concentrations were estimated using a pharmacokinetic model. We used a POD of 1 μM to estimate a plasma equivalent concentration of 414 ng/mL, which is reduced to 1.4 ng/mL after adjustment for uncertainty factors (300). In comparison, studies reported associations between serum PFOA levels and markers of hepatotoxicity in populations with median levels ranging from 0.5 to 17 ng/mL. Using a pharmacokinetic model, we estimated an equivalent dose of 0.13 ng/kg/d. In summary, the plasma level derived from in vitro studies was within the range of median plasma levels from epidemiological studies. The derived acceptable exposure level was well below existing exposure guidelines. Future work should address adjustment factors to derive acceptable exposure levels from in vitro data.
2692 **QUICK:** Quality and Usability Investigation and Control Kit for Mass Spectrometric Data from Detection of Persistent Organic Pollutants


Persistent organic pollutants (POPs) cause a significant public and environmental health concern due to their toxicity, long-range transportability, persistence, and bioaccumulation. The US Food and Drug Administration (FDA) has a program to monitor POPs in human and animal foods at ultra-trace levels, using gas chromatography coupled with mass spectrometry (GC-MS). Stringent quality control procedures are practiced within this program, ensuring the reliability and accuracy of these POP results. Due to the complexity of this program’s quality control (QC), the decision-making process for data usability was very time-consuming, upward of three analyst hours for a batch of six extracts. We significantly reduced this time by developing a software kit, written in Python, to evaluate instrument and sample QC, along with data usability. A diverse set of 45 samples were tested using our software, QUICK (Quality and Usability Investigation and Control Kit), that resulted in equivalent results provided by a human reviewer. The software improved the efficiency of the analytical process by reducing the need for user intervention, while simultaneously recognizing a 95% decrease in data reduction time, from 3 hours to 10 minutes.

2693 **Astroglial Cells and Neurotoxicity of 2,2',5,5'-Tetrachlorobiphenyl (PCB 52) and Its Metabolites**

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Exposure to polychlorinated biphenyls (PCBs) correlates with developmental neurotoxicity. Non-dioxin-like, ortho-substituted PCBs are thought to be the primary drivers of these adverse neurological outcomes, with PCB 52 being a specific congener of concern. The overall goal of our work is to determine the mechanism(s) of neurotoxicity of PCB 52, a non-dioxin-like, ortho-substituted PCB congener. Since neuronal health is dependent on astroglia, a major glial cell type in the brain, we hypothesize that astroglia play a role in mediating neurotoxicity of PCB 52 and its metabolites. C6 cells (rat glioma cell line) and primary glial cells (isolated from C57BL/6 mice) were exposed to varying concentrations of PCB 52 or two of its metabolites found in humans, namely, 2,2',5,5'-tetrachlorobiphenyl-4-ol (4-OH-PCB 52) and the corresponding sulfate (4-P-PCB 52-sulfate), for 24 hours. The C6 cells were similarly treated with three other PCB sulfates, namely, 4'-Sulfoxo-4-Chlorobiphenyl (PCB 3-sulfate), 4-Sulfooxy-3,3'-Dichlorobiphenyl (PCB 11-sulfate) and 4-Sulfooxy-2,3'-4-Trichlorobiphenyl (PCB 25-sulfate). The MTT assay was employed to determine cell viability. C6 cells were also treated with non-toxic doses of PCB 52 and its metabolites to assess effects on reactive oxygen species (ROS) generation. Results indicate that, for C6 cells and primary glia, 4-OH-PCB 52 was the most cytotoxic compound, followed by the parent compound, PCB 52. The PCB 52-sulfate was cytotoxic to C6 cells but not primary glia. This observation indicates that PCB 52, along with its metabolites, is toxic to astroglial cells with varying toxicity for the 4-OH-PCB 52. However, the three other PCB sulfates did not show cytotoxicity at any of the tested concentrations in C6 cells, indicating the importance of the structure-activity relationship in PCBs mediated toxicity. The assessment of the effect on ROS generation suggests that PCB 52 and its metabolites cause increased mitochondrial ROS even at non-toxic doses. Further studies are needed to elucidate the mechanism of toxicity of PCB 52, and its metabolites in astroglia and the role of astroglial cells in PCB mediated neurotoxicity. Supported by P42 ES013661 and R01 ES029035.

2694 **Short- and Long-Term Metabolic Effect of Persistent Organic Pollutants in Immature Mice**


Emerging evidence has identified associations between persistent organic pollutant (POP) exposure and the potential for increased risk of metabolic disorder, obesity, and diabetes. To better understand the specific mechanisms of POP-associated diseases, the short- and long-term metabolic response of immature mice exposed to two POPs with different half-life in rodents including 2,3,7,8-tetrachlorodibenzofuran (TCDF) and 3',3',4,4',5-pentachlorobiphenyl (PCB-126) were assessed using 16S rRNA gene sequencing, metagenomics, and NMR- and mass spectrometry-based metabolomics. Four weeks old male C57BL/6 wild type mice were treated with 24 µg/kg POP for 5 days. The mice were sacrificed on the day after last POP exposure (short-term) or at 13th week after POP exposure (long-term). This study demonstrated that (1) in short-term, TCDF had a greater impact on immature mice than PCB-126 with significantly higher liver oxidative stress (liver GSH/GSSG ratio decreased from 2.4 ± 0.7 [vehicle] to 0.9 ± 0.1 [TCDF] and 2.5 ± 0.9 [PCB-126]) and aryl hydrocarbon receptor (AhR) target gene expression (liver Cyp1a1 increased from 3.4 ± 2.1 [vehicle] to 1341 ± 85 [TCDF] and 896 ± 83 [PCB-126]) as well as dramatic metabolic changes including bile acid metabolism and fatty acid metabolism; (2) in long-term, TCDF-treated mice had limited significant metabolic changes that is associated with no significant changes in AhR target gene expression and undetectable TCDF levels in the bile. The liver (liver GSH/GSSG ratio decreased from 0.13 ± 0.02 µg/g [short-term] to 0.0 [long-term]); (3) in long-term, PCB-126 exposure resulted in significant higher levels of AhR target gene expression (liver Cyp1a1 increased from 1.6 ± 0.4 [vehicle] to 0.7 ± 0.3 [TCDF] and 740 ± 132 [PCB-126]) and liver lipid and macrophage profiles as well as drastic changes in fatty acid metabolism; and (4) the alteration of gut microbiota community and function were observed following TCDF and PCB-126 exposure in both short- and long-term models. These data provide new insights into the biochemical consequences of POP exposure involving the development of metabolic diseases and suggest the different role of these pollutants as disruptors of host metabolism and bacteria metabolism.

2695 **Sulfonation of Hydroxylated Bromodiphenylethers in One Marine and One Freshwater Fish Species**

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Hydroxylated bromodiphenylethers (OH-BDEs) are present in the aquatic environment from two sources, naturally produced molecules and environmental pollutants. Marine bacteria produce several methoxyated and OH-BDEs that can be taken up by marine fish and shellfish. BDEs used as flame retardants are detected worldwide in humans, animals and freshwater or marine organisms. The goal of this study was to determine the capacity of detoxification of environmental chemicals in fish. In this study, liver cytosol fractions from the freshwater fish, channel catfish, Ictalurus punctatus (n=4) and the marine fish, red snapper, Lutjanus campechanus (n=3) were used to investigate the sulfonation of three OH-BDEs (6-OH-BDE47, 2-OH-BDE68 and 4-OH-BDE68) and one bi- substituted BDE (2-OH-6-MeO-BDE68). The rates of sulfonation were measured through use of 3-phosphoadenosine-5-phos- pho-3'-sulfate (3'-PAPS). The concentration of 3'-PAPS was held at 20 µM and linear conditions for product formation with time and protein concentration establishment were set for each species. The concentrations of the OH-BDEs were varied in order to determine enzyme kinetics. In both species the enzyme kinetics fit the Michaelis-Menten equation. Mean K_m values in the red snapper ranged between 27 and 66 µM depending on substrate, and between 46 and 127 µM in the channel catfish. The highest affinity OH-BDE was 6-OH-BDE47 in the channel catfish and 2-OH-BDE68 in the red snapper. The range of V_max values was 368 to 1038 pmol/min/mg protein in the channel catfish and 92 to 495 pmol/min/mg protein in the red snapper. Comparing individual OH-BDEs between species, V_max values were higher in the channel catfish than the red snapper. This study showed that the selected marine and freshwater fish can readily sulfonate OH-BDEs derived from natural products, a process that should aid their elimination.

2696 **Long-Term Exposure to Aroclor 1260-Induced Hepatic Injury, Inflammation, Fibrosis, and Tumors in a Diet-Dependent Manner in Mice**

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Aroclor 1260, an environmental toxicant associated with numerous human diseases, was previously shown by our group to exacerbate nonalcoholic fatty liver disease (NAFLD) and disrupt hepatic energy metabolism in conjunction with high fat diet feeding (HFD) in a 12-week exposure mouse model. The current study aims to determine the long-term effects of Aroclor 1260 (8g/kg 30 weeks exposure) in NAFLD development and associated liver inflammation, injury and fibrosis. Male C57BL/6 mice were exposed to Aroclor 1260 (20mg/kg) or vehicle control via a single oral gavage and fed a low-fat diet (LFD) or HFD for 32 weeks. Physiological and metabolic assessments were performed; plasma and tissue samples were collected for further analyses. HFD-fed mice consistently exhibited increased liver oxidative stress, decreased consumption and higher organ to body weight ratios than LFD groups. The HFD groups showed elevated ALT, AST, cholesterol, and HDL and decreased triglycerides, indica-
2697 Polychlorinated Biphenyls Caused Sex-Dependent Alterations on the Hepatic Proteome That Differentially Modulate Key Processes in Toxicant-Associated Liver Disease

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Identifying sex-dependent effects resulting from pollutant exposures is fundamental in environmental health for proper risk assessment in exposed populations. Of our prior work, we demonstrated that exposure to polychlorinated biphenyls (PCBs), a class of persistent organic pollutants, led to sex-dependent outcomes in toxicant-associated liver disease in an acute exposure model. The current study aims to investigate mechanisms related to the observed sex differences using an Omics approach. Male and female C57Bl/6 mice were exposed to a mix of Aroclor1260 (commercial PCB mixture; 20 mg/kg) and PCB126 (20 μg/kg) p.o for 2 weeks. Hepatic tissue was collected for peptide measurements (LC/MS); data analysis was performed using MetaCore. Phenotypic results demonstrated that female mice were more susceptible to PCB-induced liver toxicity (steatosis, inflammation and dyslipidemia) vs. males. Proteomics analysis revealed distinct hepatic proteomes dependent on sex and exposure. Out of 3665 proteins analyzed, 262 proteins (236 up, 26 down) were significantly altered in unexposed male vs. female mice; 297 proteins (197 up, 100 down) were altered in PCB-exposed males vs. females. Overall, while both PCB-exposed males and females showed increased levels for aryl hydrocarbon receptor (AhR) targets such as Cyp1a2, female mice also exhibited increased protein levels of other AhR targets including flavin-monooxygenases. Gene ontology (GO) Process and Interaction by Protein Function (IPF) analyses revealed key processes and proteins altered in PCB-exposed females including “fatty acid metabolic process”, “fatty acid oxidation” and increased over-connectedness of transcription factors (CYP1A, SREBP1), enzymes (fatty acid transport protein 1) and lipids (low density lipoprotein). IPF analysis also showed PCB-exposed male mice displaying over-connectedness for micro-RNA (miR-132) while exposed females had increased miR-132, miR-150, miR-221 and GO processes such as “toxin metabolic process” and “dibenzo-p-dioxin metabolic process”. Taken together, the proteomics results implicated that female mice were more responsive to AhR activation and lipid dysregulation by the PCB mix which may have attributed to the observed steatosis and inflammation. Additionally, other key players such as micro-RNAs were identified that may mediate sex-dependent hepatic outcomes and warrant further investigation.

2699 Aryl Hydrocarbon Receptor Activation Contributes to Hepatic Lipogenesis through Ceramide Biosynthesis in Mice


Exposure to even low dose persistent organic pollutants (POPs) can contribute to hepatic lipogenesis and inflammation. However, the precise mechanisms in which POPs induce liver injury and dyslipidemia are largely unknown. In this study, a combination of LC-MS, 1H NMR-based metabolic profiling, RT-qPCR, ChIP-qPCR and EMSA were employed to investigate ceramides, lipids, triglyceride accumulation, as well as related gene expression and transcription. This study demonstrated that AhR activation by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure (24 μg/kg body weight/day for 5 days) induced significant elevation of hepatic ceramides, lipids, and triglycerides as well as ceramide synthesis (Smpd1, Smpd2, Smpr3, Smpr4, Sptlc1, Sptlc2, Cers2, Cers6, and Asah1) and lipogenesis-related (Srebp1c, Cidea, Elov5, Elovl3, and Sdc1) gene expression in mice. An examination of the Sptlc2 promoter (-3kb/-480) revealed the presence of two putative dioxin response elements (DREs) located at -2457 bp (DRE1) and -40 bp (DRE2). AhR promoter occupancy significantly increased following TCDF exposure for Sptlc2 (FC = 2.27, p<0.05) as determined by ChIP assay and confirmed by EMSA and cell-based luciferase reporter assays (FC = 1.30, p<0.001). These findings suggested that AhR-bound and activated the gene promoter of Sptlc2, which encodes Serine Palmitoyltransferase 2 (SPT2) that catalyzes the first, rate-limiting, step in de novo ceramide biosynthesis, leading to ceramide accumulation in the liver.

2700 Methylmercury Modulates the Cytochrome P450 1a1 and 1a2 in C57Bl/6 Mice

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During the last couple of decades, efforts have been made to study the toxic effects of individual aryl hydrocarbon receptors (AhRs) ligands such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or heavy metals typified by mercury (Hg)1+ or its metabolite, methylmercury (MeHg). However, little is known about the combined toxic effects of TCDD and MeHg in vivo. Previous reports from our laboratory and others have demonstrated that Hg1+ , by itself or in the presence of AhR ligands, such as TCDD, is capable of differentially altering the expression of various phase I and phase II AhR-regulated genes in C57BL/6J mice. Thus, the main objective of the current study was to investigate the effect of MeHg on cytochrome P450 1a1 subfamily (Cyp1a1/1a2) in C57BL/6J mice. For this purpose, mice were monocularly exposed intraperitoneally with Hg2+ or MeHg (2.5 mg/kg) or with or without TCDD (15 μg/kg) for 6 h and 24 h. Real-time PCR, Western blot analysis, 7-ethoxy-resorufin O-deethylation (EROD) and 7-methoxy-resorufin O-demethylation (MROD) assays were used to determine hepatic Cyp1a1 and Cyp1a2 mRNA, protein expression and enzymatic activity, respectively. Our results showed that Hg2+ and MeHg alone did not alter the liver Cyp1a1/1a2 expression. On the contrary, TCDD significantly induced their expression at all transcriptional levels. Interestingly, when animals were co-exposed to MeHg and TCDD, MeHg significantly inhibited the TCDD-mediated induction of Cyp1a1 and Cyp1a2 mRNA by 2.6-fold and 2-fold at 6 h, also by 5- and 2-fold at 24 h, respectively. In addition, only Hg2+ was shown to alter TCDD-mediated induction at the post-transcriptional level of Cyp1a1 protein by 4-fold and of Cyp1a1 and Cyp1a2 catalytic activity levels by 5.8- and 5-fold, respectively. In conclusion,
we demonstrate for the first time that there is a differential modulation of the TCDD-mediated induction of Cyp1a1 and cyp1a2 by MeHg. This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant RGPIN 250139-107 to A.O.S.E. MA is the recipient of Saudi Government Scholarship.

2701 The Effects of a One-Time Intraperitoneal Injection of PCB126 on the Colon Microbiome in Aryl Hydrocarbon Receptor (AhR) Knockout and Wild-Type Holtzman-Sprague-Dawley Rats

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The gut microbiome is sensitive to diet and environmental exposures. Due to its mutualistic relationship with the host body, this may lead to dysregulations and disease. Dietary exposure to dioxin-like compounds, such as PCB126, causes many adverse health effects throughout the body such as non-alcoholic fatty liver disease (NAFLD). PCB126 may also change the gut microbiota. Incorporated PCB126 may undergo enterohepatic circulation resulting in further dysregulations throughout the body. Our hypothesis was that by enterohepatic circulation, PCB126 can significantly alter the gut microbiome and that these alterations are independent of PCB126-activation of the aryl hydrocarbon receptor (AhR) of the host. We treated male and female wild-type and C57PR/Cas9-created AhR knockout Holtzman Sprague-Dawley rats with 1 IP injection of either PCB126 or corn-oil (control). Four weeks later rats were sacrificed, and fecal samples were collected from the colon. 16S rRNA sequencing was used to determine the impact of PCB126 on the colon microbiome. Microbial richness and diversity were compared between each treatment group of the same sex. The analyses showed that the overall diversity of the microbiomes and Firmicutes to Bacteroidetes ratios were not altered after PCB126 exposure in both male and female rats. However, in females, PCB126 and knocking out the AhR lead to specific taxon alterations after a one-time IP injection. These data imply that enterohepatic circulation of PCB126 can lead to microbial dysbiosis even when the route of exposure is not through ingestion. The sex differences were unexpected and need further examination.

2702 Multigenerational Impacts of Dietary Exposure to the Flame Retardant, BDE-99, in the Atlantic Killifish (Fundulus heteroclitus)

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Polybrominated diphenyl ethers (PBDEs) are a class of flame retardants that are persistent, bioaccumulative, and ubiquitous environmental pollutants linked to a variety of adverse health effects, including endocrine disruption and developmental neurotoxicity. Although maternal transfer of PBDEs has been documented, relatively few studies have investigated effects propagated across generations. Using Atlantic killifish (Fundulus heteroclitus) as a vertebrate model system, we are testing for multigenerational impacts of dietary exposure to a predominant PBDE congener, BDE-99. Adult wild-captured killifish were fed diets amended with two concentrations of BDE-99 (37.5 and 150 mg/g fish wet weight/day, for 64 days. Fish length and weight were measured at the start and end of the exposure period to monitor growth. To produce the F1 generation, exposed (F0) fish were manually strip spawned at five time points, and eggs were fertilized with subsets archived to quantify maternal transfer. At termination of the dietary exposure, the F0 adults were sampled, and tissues (brain, liver, gonads, and abdominal fat) were weighed and archived for molecular analyses. No significant differences in growth, tissue indices, reproduction, or fertilization rate were observed between treatments in the directly exposed generation. To evaluate neurological impairment in the offspring (F1 generation), locomotor activity was measured in response to alternating light/dark conditions at larval and juvenile time points. Hypoactivity was observed in some of the BDE-99 exposure lineages. At 4 months post-hatch, novel tank diving tests were conducted to assess anxiety-like behavior. Results suggest an anxiolytic effect in F1 fish parental-ly-exposed to BDE-99. Ongoing experiments will test for persistence of behavioral alterations in the F1 generation, as well as impacts propagated across generations from these parental exposures.

2703 Developmental Polychlorinated Biphenyl (PCB) Exposure Alters Voiding Physiology in Mice


Early life environmental exposures to polychlorinated biphenyls (PCBs) have been linked to alterations in the brain, but their effects on peripheral targets like the bladder are unknown. Lower urinary tract symptoms (LUTS) affect individuals of all ages and are pervasive in those with neurodevelopmental disorders, yet the underlying factors contributing to LUTS risk are not completely understood. Therefore the aim of this study was to test the hypothesis that early life PCB exposure contributes to bladder dysfunction in young adult mice. C57Bl/6J mouse dams were dosed with a PCB mix which mimics those found in human serum. Dams were dosed orally with 0.1, 0.1, 1 or 6mg/kg PCB daily for two weeks prior to mating, through gestation and lactation. Voiding function was assessed in offspring 6-8 weeks of age using void spot assay (VSA, n=17-24), uroflowmetry (n=14-24), & anesthetized cystometry (n=7-10). In vivo bladder bath assays (n=5-8) examined bladder contractility. PCB effects on voiding function were sex and dose dependent. Compared to vehicle, PCBs increased the number of small diameter (0-0.1cm) urine spots in the 0.1 & 6mg/kg PCB groups in males, & all female dose groups. Uroflowmetry testing showed that in only male mice, 0.1mg/kg PCB leads to a more drop-like void pattern instead of a stream. In mice undergoing anesthetized cystometry, PCBs had a significant overall dose effect, decreasing interval output in the 0.1 mg/kg/d & 6 mg/kg/d PCB groups compared to control. Ongoing bladder bath assays suggest that PCBs increase sensitivity to contractile stimuli via electrical field stimulation or the cholinergic agonist, carbachol, overall increasing the percent of maximal contraction response in male mice. These results support the hypothesis that PCB exposures can contribute to voiding function in mice. Overall PCB effects on voiding function included increased small diameter urine spots, drop like voids & shorter time intervals between voids. Further studies into mechanisms associated with these sex & dose-dependent effects for PCB exposure are warranted. Supported by NIH awards R01ES029537, T32ES007015, & U54DK104310.

2704 Prenatal and Postnatal Exposure to Polychlorinated Biphenyls Alter Hormone Receptor Expression in the Rat Ovary

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Endocrine-disrupting chemicals (EDCs) are known to have adverse effects on development, hormone expression, and neurological outcomes. Among the various EDCs, polychlorinated biphenyls (PCBs) were used in industrial applications until being banned in the 1970s. However, PCBs are still prevalent in the soil and water, and they persist in the food chain for humans. Although PCBs are widely known for their negative effects on the nervous system, little is known about whether they are ovarian toxicants. Thus, this study focused on whether pre- and post-conception exposures to PCBs affect gene expression in ovaries. Sprague-Dawley rats were exposed to the PCB mixture Aroclor 1221 (A1221) at 1 mg/kg/day during each of two critical windows: embryonic days 8-18 and postnatal days (PND) 1-21. Ovaries from PND8, PND32, and PND60 rats from the F1 and F2 generation were collected for qPCR analysis of differential gene expression of the progesterone receptor (Prgr), a marker of proliferation Ki-67 (Ki67), and estrogen receptor 1 (Esr1) at each age. In the F1 generation, PCB exposure did not cause statistically significant changes in the expression of in Esr1 (PND8), Prgr (PND32 and PND60), and Ki67 (PND8 and PND32). In the F2 generation, postnatal exposure to PCBs led to a significant (p ≤ 0.05) decrease in Prgr (PND8) and a borderline (0.05 < p ≤ 0.10) increase in Ki67 expression (PND32) compared to control. Collectively these data suggest that prenatal and postnatal exposure to PCBs alters the expression of key hormone receptors and a marker of proliferation in the ovary. This work is supported by NIH R01 ES02994.
Di-2-ethylhexyl phthalate (DEHP) is a chemical used commonly as a plasticizer to make polyvinyl chloride products more durable and flexible. Although exposure to DEHP has raised many health concerns due to its identification as an endocrine disruptor, it is still often used in consumer products including medical tubing, car interiors, building supplies, and children’s toys. To investigate the impact of early life exposure to DEHP on the ovary, 2-day-old piglets were administered DEHP (20 or 200 mg/kg body weight/d) or vehicle control (tocopherol-stripped corn oil) added to a sow-milk replacer for 21 days. Immediately following treatment, ovaries were harvested for histological assessment of follicle numbers and types and sera from male and female piglets were collected for measurement of steroid hormone levels. DEHP treatment did not affect the total number and percent of follicle types compared to controls. In male piglets, progesterone levels were reduced by three-fold in both treatment groups compared to controls, but testosterone and estradiol levels were not altered compared to controls. In female piglets, progesterone levels were increased by two-fold in the 20 mg/kg body weight/d treatment group and four-fold in the 200 mg/kg body weight/d treatment group compared to controls, but testosterone levels were not altered compared to controls. Thus, orally administered DEHP differentially impacts steroidalogenesis in male and female piglets, but it does not affect follicle numbers in neonatal piglets. These results suggest that early life DEHP exposure acts as an endocrine disruptor in piglet ovaries. This work was supported by NIH R01 ES028661 and NIH T32 ES007326.
Triphenyltin (TPT) is an organotin chemical used as a catalyst and biocide. TPT can stimulate cholesterol efflux in non-stereoidogenic cells. Since cholesterol is the first limiting step for sex hormone production, we hypothesized that TPT disrupts intracellular cholesterol transport and impairs steroidogenesis in ovine theca cells. We investigated TPT’s effect on cholesterol trafficking and steroidogenesis in primary cultured sheep and human ovarian theca cells. Cells were isolated, purified and exposed to an environmentally relevant dose of TPT (1 or 10 ng/ml) and/or retinoid X receptor (RXR) and liver X receptor (LXR) antagonist for 48 h (pre-luteinized cells) or 72 h (during luteinization). The expression of RXRβ and LXRβ in ovine theca cells was knocked down by shRNA. Cell cytotoxicity was assessed using an MTT assay. Steroidogenic enzymes, cholesterol transporters, and nuclear receptors expression were measured by RT-qPCR, and intracellular cholesterol, progesterone, and testosterone secretion by ELISA. In ovine cells, TPT upregulated STAR, ABCA1, and SREBF1 mRNA in pre-luteinized and luteinized theca cells (P < 0.05). TPT upregulated progesterone production (P < 0.05) but did not alter testosterone production. In human cells, both RXR and LXR antagonists blocked TPT-induced ABCA1 and STAR upregulation (P < 0.05). Upon RXRβ and LXRβ knockdown, TPT upregulated ABCA1 expression was reduced (P < 0.05). TPT’s effect on ABCA1 and SREBF1 expression was recapitulated in human theca cells. When ovine theca cells were co-exposed to TPT and tributyltin (TBT), a potent Ah receptor agonist, compared to ABCA1 and STAR, was not higher to that of either single chemical. We have demonstrated that, at an environmentally relevant dose, TPT can stimulate theca cells cholesterol transporter ABCA1 expression via RXR and LXR pathways. The upregulation of STAR that regulates cholesterol transfer into the mitochondria and SREBF for de novo cholesterol synthesis may be not antagonistic to steroidogenic hormone expression. Our findings in both, ovine and human theca cells suggest that TPT’s effect is likely a conserved mechanism of action across mammalian species. Supported by NEIHS R01 ES027863 to AV-L.

Polychlorinated Biphenyls (PCBs) are persistent environmental pollutants with endocrine disrupting properties. The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor involved in the regulation of biological responses to planar aromatic hydrocarbons. Among 209 different congeners, PCB126 is the most potent dioxin-like toxicant and AhR agonist which displays numerous adverse effects on human health, including female reproductive health. Our hypothesis is that reproductive and developmental toxicity of PCB126 in rats is AhR dependent. To test this, we created an AhR knockout (AhR-KO) model using CRISPR/Cas9. Temporal prenatals AhR-KO and wildtype (WT) Holtzman Sprague Dawley dams were used. Dams received a single IP dose of corn oil or PCB126 in corn oil on gestation day (GD) 12 and were necropsied after 6 days on GD 18. WT dams gained significantly less body weight after PCB126 exposure, but AhR-KO dams had no change. PCB126 significantly increased relative liver weights and decreased relative thymus weights in WT dams, not in AhR-KO dams. Similarly, PCB126 caused macrovesicular vacuolation and showed low glycogen in the liver of WT dams only. In addition, serum non-esterified fatty acids (NEFAs) and insulin levels were reduced in PCB126 exposed WT dams, not in AhR-KO. PCB126 also imbalanced hormones and increased serum corticosterone, reduced progesterone and decreased thyroid hormone (T4) levels in WT dams only. PCB126 not only caused hepatic and endocrine disruption in WT dams but also reduced placental weights and diameters including significant necrosis in WT dams’ placenta. PCB126 decreased the thickness of the labyrinth and the decidua layers and increased the thickness of the basal layer in the placenta of WT dams which may affect fetal development and cause hormonal imbalance in WT mothers and fetuses. PCB126 also reduced the number of viable fetuses and increased the number of non-viable fetuses, indicating fetal deaths in WT dams, not in AhR-KO. Increased low birth weights, reabsorption of fetuses (sign of miscarriage), and uterine hemorrhage were observed in only WT dams and fetuses. Therefore, all adverse manifestations were observed in WT dams and fetuses, while none were detected in AhR-KO, indicating the direct involvement of the AhR in the mediation of reproductive and developmental toxicity due to even 6 days in utero exposure to PCB126.

**2710 Involvement of the Aryl Hydrocarbon Receptor to Gestational and Developmental Toxicity of PCB126 in Rats**

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Water disinfection has reduced the incidence of waterborne diseases. However, the reaction between disinfectants and organic matter in water forms water disinfection byproducts (DBPs). Iodoacetic acid (IAA) is one DBP that has been shown to be an ovarian toxicant in vitro, but its effects on the ovaries in vivo are not well known. This study determined whether IAA exposure affects estrus cyclicity, steroidogenesis, and ovarian gene expression in mice. Adult CD-1 mice were dosed with water or with IAA (0.5, 10, 100, or 500 mg/L) in the drinking water for 35 days and estrous cyclicity was monitored for 14 days. Ovaries were collected for analysis of expression of apoptotic factors, cell cycle regulators, steroidogenic factors, estrogen receptors, oxidative stress markers, and a proliferation marker. Sera were collected to measure pregenenolone, androstenedione, testosterone, estradiol, inhibin-B, and follicle stimulating hormone (FSH) levels. IAA exposure (500 mg/L) decreased the time that the mice spent in proestrus compared to control. Further, IAA exposure decreased expression of the pro-apoptotic factor Bok (100 and 500 mg/L), the cell cycle regulator Ccnd2 (500 mg/L), and borderline decreased expression of the anti-apoptotic factor Bcl2 (10 mg/L). The pro-apoptotic factor Aimf1 (0.5 mg/L), and the steroidogenic factor Cyp19a1 (10 and 500 mg/L) compared to control. IAA exposure increased expression of the pro-apoptotic factors Bax and Aimf1 (500 mg/L), the anti-apoptotic factor Bcl2 (100 mg/L), the cell cycle regulators Ccna2, Ccnb1, Ccnb1, and Cdk4 (500 mg/L), and the theca cell growth hormone Cyp11a1 (100 mg/L), the cell cycle regulator Ccna2, and the anti-apoptotic factor Bcl2 (500 mg/L). This phenomenon occurred in exposed vessels when measuring their reactivity to PGI2 significantly increased for nano-TiO2 during gestation alters uterine microvascular adaptation. Uterine blood flow alterations are influenced by endothelial and vascular smooth muscle signaling. The cyclooxygenase metabolites prostacyclin (PGI2) and thromboxane (TXA2) are important mediators of this process. In a healthy pregnancy, PGI2 and TXA2 work together to regulate uteroplacental blood flow. Altered responsiveness to these metabolites may impair fetal development. We hypothesize that maternal inhalation exposure to nano-TiO2 during gestation alters uterine microvascular PG12, and TXA2 reactivity. Pregnant rats were assigned to filtered air or nano-TiO2 exposed groups. Rats were exposed 6 hrs/day for 6 days on gestational day (GD) 10-19 to non-toxic aerosols generated with Evonik P25 (concentration= 12±0.5 mg/m3); cumulative lung burden= 525±16 µg; primary particle size= 21 nm; zeta potential=-57 mV). On GD20, rats were euthanized (24-hrs post final exposure). Age, body mass, litter size or pup distribution in the uterine horn were not different between groups. Nano-TiO2 exposure decreased (p<0.05) placental weight compared to controls (1483±64 vs 1978±74 pg/mL), but did not alter testicular weight (0.7±0.01 vs 0.7±0.01 g). Circulating serum level of TXB2, the stable TXA2 metabolite, was decreased (p<0.05) in nano-TiO2 exposed groups compared to controls (1483±63 vs 1978±74 pg/mL). Uterine arterial responsiveness to PG12 and TXA2, had a unique response within nano-TiO2 exposures. A subgroup presented with augmented responses, when this was accounted for reactivity to PG12, significantly increased for nano-TiO2 exposed groups compared to controls (68±15% vs 36±7% maximal response). This same phenomenon occurred in exposed vessels when measuring their reactivity to TXA2, in which augmented exposed vessels produced a hyper contractile response compared to controls (-22±5% vs -10±2% maximal response). These results lead us to propose that gestational nanomaterial inhalation exposure alters COX metabolites in pregnancy adaptations. Future work will focus on contributions of PG12 and TXA2, in this mechanism. Support: ES015022 (TRN); ES031253 (SH).
2713 Maternal Gestation Engineered Nanomaterial Exposure Impairs Vascular Reactivity, Estrogen, and Fertility in F1 Females

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We have reported that maternal inhalation exposure to nano-titanium dioxide (nano-TiO2) during gestation severely reduces estrogen and compromises placental efficiency at gestational day (GD) 20 in Sprague Dawley (SD) rats. Perturbations to the normal gestational milieu neonates are subjected to during development has been shown to significantly contribute to the developmental origins of health and disease. The objective of this study was to determine the effects of gestational exposure on vascular reactivity, fertility, and pregnancy outcomes. Female, SD rats were housed in the West Virginia University Inhalation Facility under a regulated temperature and 12:12 hour light-dark cycle. Dams of F1 offspring were randomly assigned to the sham-control or nano-TiO2 exposure groups and acclimated for 48-72-hours before mating. Inhalation exposures lasted for 6 days after GD 10 to decrease animal stress. Pregnant rats were exposed to an average target concentration of 12 mg/m3. This exposure paradigm (12 mg/m3, 6h/exposure, 6 days) produced a calculated lung burden of 525±16 μg. Dams gave birth and F1 females were weaned 21 days postnatally. F1 females were bred at 8 weeks and sacrificed at GD 20. Rats born to exposed dams experienced increased time to conception, requiring at least two matings prior to successful conception. Dams exposed to nano-TiO2 also had significantly reduced litter sizes (10.2±0.68) with decreased number of males (4.4±0.38), compared to sham-controls (12.7±0.96 and 6.8±0.83 respectively). Litter size was also significantly reduced in F1 females born to exposed dams compared to F1 females born to sham-control dams (9.5±0.57 vs. 11.5±0.68). Uterine arteries from F1 females born to exposed dams showed increased vasoconstriction to phenylephrine (58.6±10.2%) and kisspeptin (75.1±14.2%) compared to uterine arteries of F1 females born to sham-control dams (28.6±2.6% and 92±16.2%, respectively). Estrogen concentrations on GD 20 were also significantly reduced in F1 females born to exposed dams (12.1±3.1 pg/ml) vs. sham-control (29.3±8.8 pg/ml) dams. These results provide evidence that maternal nanomaterial inhalation exposure during gestation leads to multigenerational vascular, endocrine and reproductive consequences. Support: ES015022 (TN) ES031253 (SH).

2714 Effects of Fractions of Saccharum officinarum Molasses and Refined Sugar on Reproductive Functions of Male Wistar Rats

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Previous reports on the anti-androgenic activities of refined sugar has led to the search for safer substitutes. Saccharum officinarum molasses (SOM) is a by-product obtained during refined sugar production. Having its own sweetness, SOM is widely used as a sweetener and is becoming a popular alternative product obtained during refined sugar production. Having its own sweetness, SOM is widely used as a sweetener and is becoming a popular alternative for sugar. This study investigated the effects of SOM and compared them to those of refined sugar on male reproductive functions. Saccharum officinarum molasses (Blackstrap) was fractionated to obtain aqueous (SOMAF) and methanol (SOMMF) fractions, and subjected to phytochemical screening. Twelve groups (n=5) of adult male Wistar rats received, distilled water (Control); 0.8, 2.5, 7.9 g/kg SOM; 0.0064 g/kg sugar (Dangote’); 0.0064 g/kg sugar+7.9 g/kg SOM; 0.6, 2.0, 6.4 g/kg SOMAF and 1.0, 3.2, 10.0 g/kg SOMMF, respectively. Daily administrations were via oral gavage for a period of eight weeks. Sperm profile and histogram of testes and epididymides were assessed by microscopy. Sperm phenotype was measured using Enzyme Linked Immunosorbent Assay. Testicular malondialdehyde was assayed by spectrophotometry. Data were analyzed using ANOVA at p ≤ 0.05 significance. Sperm count and viability reduced with 7.9 g/kg SOM, Sugar, 3.2 and 10.0 g/kg SOMAF, 2.0 and 6.4 g/kg SOMAF. Abnormal sperms increased with 7.9 g/kg SOM, Sugar, 2.0 and 6.4 g/kg SOMAF. Testosterone level reduced with 6.4 g/kg SOMAF. Testicular malondialdehyde increased with SOM, 3.2 and 10.0 g/kg SOMMF as well as 6.4 g/kg SOMAF. There were histological anomalies in the seminiferous tubules and epididymal ducts of 7.9 g/kg SOM. Sugar and SOMAF-treated rats. Saccharum officinarum molasses altered testicular and epididymal integrity via lipid peroxidation, hence reducing androgen level and sperm quality in male Wistar rats.

2715 Phthalate Metabolites Act through PPAR Nuclear Receptors in the Mouse Ovary


Widespread use of phthalates as solvents and plasticizers leads to human exposure through ingestion, inhalation, and dermal contact. The mechanisms by which phthalate metabolites act as ovarian toxicants are not fully understood. Thus, this study tested the hypothesis that phthalate metabolites (MEHP, MiNP, and MBzP) act through peroxisome proliferator-activated receptor (PPAR) nuclear receptors in mouse antral follicles and granulosa cells in vitro. Antral follicles were isolated from CD-1 mice and cultured with MEHP for 96 hours. Phthalate treatments from 0.4-400 μM were compared to DMSO vehicle control. Growth of follicles in culture was monitored every 24 hours. Following culture, total RNA was extracted and reverse transcribed. Real-time PCR was then performed for genes known to be directly regulated by the PPARy nuclear receptor. The highest treatment group led to significant increases in expression of the PPARy target Cdkn1a relative to control. These increases in expression were inhibited by co-treatment with the PPARy inhibitor T0070907. Primary granulosa cells were also isolated from CD-1 mice, expanded, and treated for 24 hours with MEHP, MiNP, or MBzP. The highest treatment groups led to significant increases in expression of the PPARy target Fabp4 relative to control. These increases were inhibited by co-treatment with T0070907. Primary granulosa cell cultures were also transfected with a DNA plasmid containing lucfcerase expressed under the control of a consensus PPAR response element. MEHP, MiNP, and MBzP all caused a dose-dependent response in expression of lucfcerase; thus, indicating the presence of functional endogenous PPAR receptors in the granulosa cells that respond to phthalate metabolites. Collectively, these data suggest that the individual phthalate metabolites may act through PPAR nuclear receptors in the ovary. Supported by NIH T32 ES007326 and RO1 ES028661.

2716 Transcriptional Profiling of the Response to the Trichloroethylene Metabolite 5-(1,2-dichlorovinyl)-L-Cysteine Revealed Activation of the elf2a/ATF4 Integrated Stress Response in Two In Vitro Placental Models

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Trichloroethylene (TCE) is an industrial solvent and widespread environmental contaminant. Although TCE exposure is prevalent, epidemiological studies of TCE exposure associations with adverse birth outcomes are inconclusive. The TCE metabolite 5-(1,2-dichlorovinyl)-L-cysteine (DCVC) exibits toxicity in a placental cell line. In the current study, genome-wide gene expression and gene set enrichment analyses were used to identify novel genes and pathway alterations in human placental villous explants and the HTR-8/SVneo human trophoblast cell line treated with DCVC at concentrations relevant to human exposures. In the cells, time- and concentration-dependent effects were observed, as evidenced by the magnitude of altered gene expression after treatment with 20 µM DCVC versus 10 µM, and 12-h versus 6-h of treatment. Comparing the two models for transcriptional response to 12-h 20 µM DCVC treatment, no differentially expressed genes reached significance in villous explants, while 301 differentially expressed genes were detected in HTR-8/SVneo cells compared with non-treated controls (FDR<0.05 + LogFC>0.35 [FDR<1]). GSEA revealed five upregulated enriched pathways in common between explants and cells (FDR<0.05). Moreover, all 12-h DCVC treatment groups from both models contained upregulated pathways enriched for genes regulated by the ATF4 transcription factor. The overrepresentation of ATF4 regulation of differentially expressed genes indicated activation of the integrated stress response (ISR) - a condition triggered by multiple stress stimuli. DCVC-induced ISR activation was confirmed by elevated elf2a phosphorylation, ATF4 protein concentrations and decreased global protein synthesis in HTR-8/SVneo cells. This study identifies a mechanism of DCVC-induced cytotoxicity by revealing the involvement of a specific stress signaling pathway.
Phthalates are used in a variety of products, and are environmental contaminants that have been linked to several potential health concerns. The widespread distribution of phthalates in the environment, and their detection in the vast majority of the general population, confirm broad human exposure to this class of chemicals. To elucidate the risks of phthalates among vulnerable populations, such as pregnant women and the developing fetus, a systematic review was performed to evaluate the association between phthalate exposure during pregnancy and maternal and perinatal health outcomes. MEDLINE, EMBASE, PUBMED, CINAHL, and POPLINE were searched to identify observational studies that reported on the following outcomes: birth weight (BW), head circumference (HC), gestational age (GA), preterm birth (PB), APGAR scores (AS), intrauterine growth restriction (IUGR), pregnancy-induced hypertension (PIH), pre-eclampsia (PE), and gestational diabetes mellitus (GDM). Articles were initially screened by title and abstract, and full-texts of potentially pertinent articles were retrieved. Hand-searching of reference lists and citation tracking were conducted to supplement the primary search. In addition, articles that were recommended were also considered. Studies included in this systematic review were evaluated using a modified Downs and Black quality assessment tool. Twenty-four articles met the inclusion criteria. Most studies retrieved reported on BW, HC, GA, and PB. One article reported on AS, IUGR, PE, and GDM. No studies investigating PIH were identified. Studies assessed the ratio LC50/EC50 and PB yielded conflicting results; however, among the significant findings, the majority of the results suggest that phthalates may decrease BW and GA, and increase HC and PB. Studies evaluating AS, PE and GDM yielded non-significant findings, whereas the study on IUGR reported a significant positive association with phthalate exposure. Overall, poor correlation between reproducible associations between perinatal outcomes and decreased BW and GA, and increased HC and PB.-Inconsistencies in findings were observed among the various reports, possibly due to heterogeneity in study populations, exposure characterization and statistical analyses methodologies and data reporting in these studies.

2719 The Role of Aryl Hydrocarbon Receptor in Mediating the Effects of Mono(2-Ethylhexyl) Phthalate in Ovarian Antral Follicles

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Phthalates, like di(2-ethylhexyl) phthalate (DEHP), are pervasive environmental toxicants used in the manufacturing of numerous consumer products, medical supplies, and building materials. DEHP is metabolized to mono(2-ethylhexyl) phthalate (MEHP). MEHP is an endocrine disruptor that adversely affects folliculogenesis and steroidogenesis in the ovary, but its mechanism of action is not fully understood. We explored the role of aryl hydrocarbon receptor (AhR) in mediating these effects by culturing mouse ovarian follicles with MEHP (0-400μM) and the AhR antagonist CH223191 (1μM). MEHP treatment reduced follicle growth over a 96 hour period, and this effect was partially rescued by co-culture with CH223191. MEHP exposure alone increased expression of known AhR targets, cytochrome P450 (CYP) enzymes Cyp1a1 and Cyp1b1, and this induction was blocked by CH223191. We also observed changes in steroid hormone concentrations in the culture media from follicles exposed to MEHP. Notably, MEHP reduced media concentrations of estrone and estradiol compared to control. This observation is consistent with elevation of the estrogen metabolizing enzymes Cyp1a1 and Cyp1b1 following MEHP exposure. MEHP-mediated reduction of estradiol and estrone was mitigated by co-culture with CH223191. We also observed differential expression of estrogen regulated target genes like insulin-like growth factor 1 (Igf1), insulin-like growth factor-binding protein 4 (Igfbp4), and peroxisome proliferator-activated receptor gamma (Pparg) following exposure to MEHP compared to control. The role of AhR in mediating these effects was further supported by co-culture with the AhR antagonist CH223191.

2720 Prenatal Exposure to Arsenic: Effects on Immune Signaling in the Heart

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Arsenic is a harmful immunotoxicant which significantly affects more than 200 million people globally. Specifically, research points to the role of arsenic as a developmental toxicant, critically affecting fetal development and immune development in particular. Furthermore, existing evidence points to the vital role which specific macrophage and leukocyte functions play in cardiac development, composition, and function during gestation. However, the effects of arsenic on signaling and gene expression in the developing heart are insufficiently understood. Therefore, we hypothesize that chronic arsenic exposure during pregnancy alters signaling and gene expression in the developing heart. To study gene expression during fetal heart development, C57Bl/6 mice were preconceptionally and prenatally exposed to 0 or 100 μg/L sodium (meta) arsenite in drinking water. Fetuses were sacrificed and heart tissue samples were collected at gestation day 18. RNA was isolated from whole fetal hearts and evaluated using an Agilent 4K expression microarray followed by data cleanup and analysis on RStudio. mRNAs that were found to be significantly different between treatments (p<0.01) were queried in the String Database (string-db.org) to create a protein-protein interaction network and identify significantly enriched biological pathways. The String Map data demonstrates that the top three enriched pathways include: Immune System Process, Immune Response, and Immune Effector Process. Of the top 10 pathways, 8 are related to immune system. ARCHS4 is being used to collect relevant gene information such as related biological processes, phenotypes, and KEGG pathways. Using this information, PubMed is being searched for literature demonstrating relationships between altered gene expression with heart function and arsenic exposure. Further investigation is being conducted to identify the potential mechanisms by which these immune pathways are being dysregulated during fetal heart development. As expected, this research could reveal potential mechanisms driving cardiac deficits caused by arsenic exposure. Findings could guide research into intervention strategies for reducing long-term impacts of prenatal arsenic exposure. Supported by NIEHS R00ES024808 (FS) and T32ES028661.
Plastics are everywhere around us. Unfortunately, when disposed, these ubiquitous products are unable to degrade completely. They instead fragment into smaller pieces, producing micro- and nano-sized plastics. Previous work has demonstrated that nanosized particles are significantly more toxic than larger microparticles after inhalation. Unfortunately, studies evaluating nanoplastic toxicity are limited and studies evaluating fetal health after maternal nanoplastic exposure are nonexistent. In this study, we assessed rat fetal development and growth patterns based on anatomical uterine positioning (ovarian, middle, and vaginal) within the uterine horn after maternal pulmonary exposure to nanoplastyrene particles (NP) late in gestation. Pregnant rats (n = 14-16) were exposed to NP (2.64 x 10^14 particles) through intratracheal instillation (300 µL) at gestational day (GD) 19 and sacrificed on GD 20, along with a sterile saline control group. The anatomical uterine positioning of the fetuses within the uterus was identified, and the fetal and placental weights were measured and recorded. We identified that the rat fetuses of mothers exposed to NP during gestation were significantly smaller than control (2.68 g ± 0.04 vs. 2.54 g ± 0.03). This outcome seems to be driven by pups in the middle of the uterine and right uterine, wherein exposed pups were significantly smaller than control (middle of the uter 2.69 ± 0.04 vs. 2.53 g ± 0.05, right uter 2.66 g ± 0.04 vs. 2.50 g ± 0.04). However, a significant difference between controls and exposed was not observed in the placental weights (0.47 g ± 0.01 vs. 0.44 g ± 0.01). Additionally, fetuses in the vaginal end have the highest average weight in either the control or exposed group from the three positions analyzed, although this is not yet to significance. The results found in this study further expand our knowledge on the developmental toxicity of nanoplastics and help to achieve more accurate conclusions from reproductive toxicological studies that utilize rodent models. Future studies should focus on time and dose-dependent experiments that better simulate day-to-day exposures to obtain more environmentally relevant results. Supported by: NIH-R00-ES024783; T32-ES007148; P30-ES005022; R25-ES020721, and ASPET-SURF.
Phthalates are a group of chemicals used as additives in various consumer products, medical equipment, and personal care products. Phthalates and their metabolites are consistently detected in humans, indicating widespread and continuous exposure to multiple phthalates. Thus, environmentally relevant mixtures of phthalates and phthalate metabolites were investigated to determine the effects of phthalates on the function of the ovary during the neonatal period of development. Neonatal ovaries from CD-1 mice were cultured with either DMSO (vehicle control), phthalate mixture (0.1–100 µg/mL), or phthalate metabolite mixture (0.1–100 µg/mL). The phthalate mixture was composed of 35% diethyl phthalate, 21% di(2-ethylhexyl) phthalate, 15% dibutyl phthalate, 15% diisononyl phthalate, 8% diisobutyl phthalate, and 5% benzylbutyl phthalate. The phthalate metabolite mixture was composed of 37% monoethyl phthalate, 19% mono(2-ethylhexyl) phthalate, 15% monobutyl phthalate, 10% monoisononyl phthalate, 10% monoisobutyl phthalate, and 8% monobenzyl phthalate. After 96 hours of culture, ovaries were harvested for histological analysis of folliculogenesis, gene expression analysis of cell cycle and apoptosis regulators, and immune staining for cell proliferation and apoptosis. The metabolite mixture significantly decreased the percentage of abnormal follicles (100 µg/mL) compared to controls, suggesting decreased atresia. The metabolite mixture also significantly increased the expression of cell cycle inhibitors (10 and 100 µg/mL) and the anti-apoptotic factor Bcl2 (10 µg/mL) compared to controls. The phthalate mixture did not significantly alter gene expression or follicle counts, but ovaries exposed to the phthalate mixture (0.1 µg/mL) exhibited significantly increased apoptosis as revealed by DNA fragmentation staining. Overall, these data show that parent phthalates and phthalate metabolites differentially impact ovarian function. The first study to describe the impact of phthalate metabolites on ovarian development during the sensitive neonatal period and confirms the role that metabolism plays in phthalate toxicity. Supported by NIH R01ES028661, NIH T32ES007326, and NIH K99ES031150.

Phthalate diesters are a class of synthetic chemicals found in many consumer goods including plastic food containers, blood bags and medical tubing, as well as personal care products. Phthalates have been shown to negatively impact the female reproductive tract and are metabolized in the body to mono- and diester phthalates and their metabolites. Many xenobiotics are known to cross the blood-testis barrier (BTB) to elicit effects on developing germ cells. The present study aimed to investigate the effect of MC-LR on male reproductive health with future plans to investigate acetaminophen, aspirin, and ibuprofen. Here, we report that acute naproxen treatment reduces in vitro germ cell viability and induces cell cycle arrest. Together, these results suggest that short term naproxen administration may negatively impact human spermatogenesis. However, additional experiments are required to understand how prolonged use of naproxen or other NSAIDs affects male fertility.

Over the last several decades, semen parameters, including sperm counts, have rapidly declined. While this decline remains largely unexplained, one possible contributing factor is the widespread and increasing use of the counter (OTC) analgesics, such as non-steroidal anti-inflammatory drugs (NSAIDs). Recent studies have linked NSAID usage with declines in male fertility, but the mechanisms by which these NSAIDs impact fertility are largely unknown. Using an established stem cell-based human spermatogenesis model, we assessed the reproductive toxicity potential of acute exposure to naproxen, a common NSAID, under physiologically relevant concentrations, with future plans to investigate acetaminophen, aspirin, and ibuprofen. Here, we report that acute naproxen treatment reduces in vitro germ cell viability and induces cell cycle arrest. Together, these results suggest that short term naproxen administration may negatively impact human spermatogenesis.
several indazole carboxylic acid analogs with potent anti-spermatogenic activity in rodents but have not been approved for human use. Although these compounds have been shown to accumulate in rodent SCS, the mechanism of entry has not been well-documented. This study was designed to determine whether human transporter-mediated processes are involved in the uptake of H2-gamendazole and four related analogs into human SCS. Five-minute drug uptake studies were performed in a immortalized human testis cell line (hT-SerC) developed to study substrate-transporter selectivity and kinetics using liquid chromatography-tandem mass spectrometry (LC-MS/MS). To identify the responsible uptake transporter(s) for H2-gamendazole and the four analogs, the compound of interest was co-incubated with known transporter inhibitors or competitive substrates to outcompete H2-gamendazole uptake. H2-gamendazole and three related analogs followed Michaels-Menten kinetics of transport into hT-SerCs, suggesting these compounds use a transporter-mediated entry process. One analog (JWS-2-176) had poor penetration into hT-SerCs and the negligible amount that remained in the samples was attributed to passive diffusion. H2-gamendazole uptake was inhibited by >50% in the presence of 1 mM indomethacin, 1 mM diclofenac, or 150 µM RC-MC-100 (another related analog). Interestingly, the organic anion NSAIDs with known reductive toxicity, indomethacin and diclofenac, were also found to be transported into hT-SerCs. Moreover, the uptake of indomethacin into hT-SerCs was competitively inhibited by co-incubating with diclofenac and vice versa. No significant reduction in H2-gamendazole uptake was observed when co-incubated with organic cation transporter substrates or inhibitors such as atroline, carbamazepine, and metformin, and MPP+. These data suggest that an organic anion transporter may be responsible for transporting H2-gamendazole into human SCS. Altogether, identification of the transporters involved in the flux of indomethacin, diclofenac, H2-gamendazole and its analogs may provide insight into the selectivity of drug disposition across the human BTB to understand and overcome the pharmacokinetic and pharmodynamic difficulties presented by the BTB.
Polycyclic aromatic hydrocarbons, such as benzo(a)pyrene (BaP), are products of incomplete combustion of organic materials. Exposure to PAHs during ovarian development causes premature ovarian failure in rodents. Primordial germ cells (PGCs), embryonic precursors of oocytes, arise in the mouse yolk sac at 7.25 days postcoitum (dpc). PGCs proliferate before and after arriving at the gonadal ridge on 10.5 dpc and begin entering meiosis at 13.5 dpc. Now oocytes, they arrest in meiotic prophase I beginning at 17.5 dpc. This finite oocyte pool is the primary determinant of female fertility and reproductive lifespan. Over the course of a female’s reproductive lifespan, oocytes will grow in their follicles with the help of supportive granulosa cells, acquiring developmental competence. We have shown that in utero exposure of female mice to 2 or 10 mg/kg/day BaP during a dosing window that spans the mitotic and meiotic stages of PGC development results in depleted follicular numbers postnatally. What has been less explored is how BaP exposure in utero impacts the quality of surviving mature oocytes. We hypothesized that mature oocytes exposed to BaP in utero will have increased mitochondrial dysfunction observed by decreased mitochondrial membrane potential, increased mitochondrial superoxide production and decreased mitochondrial association with lipid droplets. We orally dosed timed-pregnant female mice to 0 or 2 mg/kg/day of BaP in oil from 6.5-11.5 dpc. F1 females were superovulated between 35-45 PND and various endpoints of oocyte mitochondria function were observed using confocal microscopy. In our preliminary data, we observed that mature F1 oocytes exposed to BaP in utero showed decreasing trends in mitochondrial membrane potential (P=0.081) and a significant decrease in mitochondrial association with lipid droplets (P=0.003), suggesting a decrease in fatty acid beta-oxidation, an important source of ATP for the mature oocyte. To further support this, we observed a significant decrease in lipid droplet content in mature exposed oocytes (P=0.02). However, we observed no difference in mitochondrial superoxide production with exposure. These results suggest that oocytes exposed to BaP in utero may be able to recover some mitochondrial dysfunction, however, there seems to be a decisive shift in the metabolism of the oocyte, possibly contributing to reduced oocyte developmental competence. This research was supported by NIH R01ES020454 to UL and UCOP TDRP T30DT0816 to KM.

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Sex-dependent differences in placental and fetal cadmium toxicity in mice


Cadmium (Cd), a ubiquitous heavy metal and environmental toxicant, is found at measurable levels in almost all pregnant women. Prenatal exposure to Cd causes fetal growth restriction likely due to disruption of placental nutrient transfer. Emerging epidemiological data suggest that the developmental toxicity of cadmium may differentially affect male versus female offspring. In this study, we sought to determine whether fetal sex impacts placental and fetal responses to Cd following in utero exposure. A single dose of saline vehicle (5 µg/kg) or CdCl2 with up-regulation of key glucose metabolic enzymes, hexokinase, and transporters may be a compensatory mechanism to restore growth of male fetuses following Cd exposure. Additional studies are underway to determine the mechanisms by which Cd causes a sex-dependent impact on fetal nutrition and growth in mice. Supported by F31ES032319, RO1ES029275, T32ES007148, and P30ES005022.

Reevaluating the reproductive toxicity of Cr(VI): Assessing the Prop 65 MADL

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Chromium is a naturally-occurring trace element found in rocks, animals, plants, and soil, and primarily exists in three separate oxidation states, Cr(0), Cr(III) and Cr(VI). The metal is used in various industries including leather tanning, as well as an agent to limit corrosion. On February 27, 1987, Cr(VI) was added to the Prop 65 list as an identified carcinogen via the labor code based on evidence from IARC regarding carcinogenicity following inhalation exposure. In 2008, the metal was also identified as a developmental and reproductive hazard by the Developmental and Reproductive Toxicant Identification Committee (DARTIC) of the Office of Environmental Health Hazard Assessment’s (OEHHA) Science Advisory Board. At the time, the DARTIC determined that the maximal allowable dose level (MADL) for Cr(VI) was 8 µg/day based on the Murthy et al., (1996) study which reported ovarian toxicity in mice. However, no quantitative measures of ovarian toxicity were found to be significant. Thus, the purpose of the current analysis was to evaluate the current weight of evidence of Cr(VI) reproductive toxicity to determine 1) if Cr(VI) was a female reproductive hazard, and 2) if more recent data could be identified to derive a revised Cr(VI) MADL. Interestingly, one study found that exposure of up to 17.7 mg Cr(VI)/kg/day did not significantly affect ovarian weights, follicular count or follicular degeneration in mice. The findings from Thompson et al., (2020) were consistent with the observations from the NTP studies which have evaluated reproductive organs following oral ingestion of Cr(VI). Utilizing data from Thompson et al., (2020) would result in an MADL roughly 5x the current MADL derived using data on the same endpoint (ovarian toxicity in mice). However, it is worth noting that in the Thompson et al., (2020) study there was no evidence of ovarian toxicity in mice at any dose, even at doses exceeding the MTD. Hence, it’s possible that any future MADL derivation may want to consider alternative reproductive health endpoints (aside from ovarian toxicity).
The novel respiratory coronavirus 2 (SARS-CoV-2) has propagated throughout the world at an unprecedented speed, leaving many health organizations and national governments across the world with little time to aptly react to this ensuing COVID-19 pandemic. The gravitas of the situation is undeniable. Over 45 million people worldwide and 9 million in the USA were afflicted by the virus. It has become imperative to properly test the efficacy and safety of any potential curative treatments in a thorough yet expedient fashion. The antiviral Remdesivir has become the frontrunner drug for the treatment of COVID-19 patients. Given the uncertainty of reports available regarding Remdesivir effect on male reproductive health, we set out to accomplish a comprehensive study delineating Remdesivir toxicity with a central emphasis on spermatogenesis. Using the C57black/6N mice and administering the drug via intravenous (IV) or Intraperitoneal (IP) injection, we measured the mouse dose response including histopathological evaluation of testes and collected organs, sperm examination, and utilized LC/MS/MS analysis to investigate toxicodynamics. The dose groups included 150, 830, 2490, or 4980 μg/kg/day or saline or Sulfa butylether-β-Cyclodextrin Sodium Salt (SBEDC) controls. These doses were the day 1 loading doses, which were followed by 9 daily treatments of half of the loading dose. Assessments were made on the last day of treatment to assess toxicodynamics and immediate toxicity, and following 35 days of recovery which allows for a full round of spermatogenic recovery. We show a lack of system toxicity and Remdesivir-mediated decreased sperm concentration (SBCD) in the liver, lung, kidney, epididymis and testis. Mild kidney histopathology was only observed in the highest dose group. A trend in decreasing sperm count was detected in the IV dosed cohort only all assessments for sperm morphology, integrity and viability appeared to be within an acceptable range. Remdesivir appears to be safe in all manners observed in mice at a wide range of doses.
Previously, we demonstrated that exposure to some dioctyl-phthalate esters (PDEs) during sexual differentiation disrupts fetal testis testosterone production (T Prod) and gene expression (ins3 and others) in a dose related manner. The objectives of the current project were to expand the number of test compounds including PDEs, PE alternatives, pesticides, and drugs and to relate these fetal testes alterations to adverse effects in the reproductive tract of the male offspring. The pesticides included in this study are known to disrupt T Prod and those that do not. We also included acetaldehyde (paracetamol), which reportedly reduces fetal T Prod like the PDEs, and several PPAR agonists including hexafluorpropylene oxide-dimer acid (HFPO-DA or GenX), pircinic acid (WI 14643), and clofibrate because it has been hypothesized that the PDEs disrupt male sex differentiation via PPAR receptors. We found that PDEs that disrupt T Prod also disrupted tests mRNA expression for about 35 genes related to sterol transport, T and ins3 hormone syntheses, and lipoprotein and cholesterol synthesis. PDEs had little or no effect however on mRNA expression for genes in PPAR pathways in the fetal liver whereas the three PPAR agonists induced the expression of mRNA for multiple PPAR pathway genes without reducing T Prod. Lirunon, prochloraz, 4-methylidazole, and bisphenol C reduced T Prod; whereas acetaminophen, vinclozolin, flutamide, DlNCH, DPH, and hexacanoa had no effect on T Prod. Dexamethasone reduced T Prod but only at dosage levels that also induced maternal weight loss. In summary, PDEs that disrupt T Prod act via a novel AOP including down regulation of mRNA for genes involved in fetal endocrine function and cholesterol synthesis and metabolism. This profile was not displayed by PDEs that did not reduce T Prod, PPAR agonists or the other chemicals. We also demonstrated that the reductions in fetal T Prod in utero could be used quantitatively to predict the doses that produce adverse reproductive tract effects in male offspring. This abstract does not reflect US EPA policy.

**Reference:**


**2743** Prenatal Exposure to an Environmentally Relevant Phthalate Mixture Alters Folliculogenesis in the F1 Generation of Adult Female Mice

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Phthalates are a family of chemicals that can be found in plastic and personal care products used by consumers every day. Phthalates are also known endocrine-disrupting chemicals that have been shown to directly target the ovary, disrupt female reproduction. In previous studies, an environmentally relevant phthalate mixture has been shown to affect female reproduction in a transgenerational manner. Despite this knowledge, it is still unknown whether the phthalate mixture affects folliculogenesis. Folliculogenesis is an important process for the development of ovarian follicles and successful fertility. Thus, the focus of this study was to understand the effects of the mixture on this process, starting with the F1 generation. Using mice as our model, we tested the hypothesis that prenatal exposure to a phthalate mixture will adversely affect folliculogenesis in the F1 generation. Pregnant dams (F0 generation) were orally dosed with control or a phthalate mixture (20 µg/kg/day-500 mg/kg/day) daily from gestational day 10 to birth, and the adult F1 females were the products of these dams. For each treatment group, the ovaries were collected at postnatal day 60 in the F1 generation, fixed, embedded, sectioned, stained, and examined using a microscope to count the different follicle types. The follicles specifically counted in this study were primordial, primary, preantral, antral, and atretic follicles. Raw counts were analyzed to observe if the mixture affected follicle numbers, and the percentages of follicles were examined to observe changes in folliculogenesis, or the shift in the follicle pool. The results indicate that the mixture at 200 and 500 mg/kg/day significantly increased atretic follicles compared to the control. Furthermore, the mixture at 20 µg/kg/day borderline increased the percentage of primordial follicles and decreased the percentage of antral follicles compared to the control. The results suggest that exposure to an environmentally relevant phthalate mixture could lead to higher incidence of atresia in antral follicles. Moreover, these data suggest that exposure to the phthalate mixture may decrease the transition of immature follicles into more mature follicle types. Supported by NIH R01ES032163.

**Reference:**


Ectopic growth of normal cells or debris originating from endometrium, the inner layer of female reproductive tube leads to endometriosis. The main symptoms are dyspareunia, dysmenorrhea, and sometimes chronic pelvic pain. Unfortunately, it has no cure or treatment, only medication for symptoms relief or surgical removal. Endometriosis causes estrogen imbalance leading to metabolic dysregulation and subsequent change in immune response. Our study aims to investigate the metabolic alteration mediated by genetic dysregulation and immunological response in endometriotic mice. Balb/c mice were ovariectomized (OVX) 7 days before intraperitoneal transplanted with endometrial tissue from OVX donors to OVX (OVX+END) or intact (Naive+END) recipients. Peritoneal exudate was collected 2 weeks post transplantation for single cell RNA sequencing (scRNA-seq), staged to determine the phenotype of infiltrating cells by flow cytometry and exposed to microarray in silico analysis. Results revealed a significant increase in the number of exudate cells and natural killer (NK) cells as well as a decrease in myeloid derived suppressor cells (MDSCs) in endometriotic mice. scRNA-seq results showed significant increase in the expression of a group of genes that increases the metabolic activity of T-cells and macrophages such as phosphatidyl-inositol-4,5-bisphosphate 3-kinase catalytic subunit delta (PIK3cd) and lactide dehydrogenase A (Ldha) and subsequent increase in the activation of these immune cells in OVX+END mice in comparison with OVX+VEH mice. Furthermore, mass spectrometry analysis of the blood of the study groups showed significant increases in glucose metabolites in OVX+END in comparison to OVX+VEH. These results were confirmed with real time metabolism analysis done by Seahorse system to show that the peritoneal infiltrating T cells taken from endometriotic mice produced significantly higher ATP than the non-endometriotic mice (naive or OVX+VEH). Collectively, endometriosis

**Reference:**

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changed estrogen metabolism and metabolic profile of the immune cells that led to increased immunotoxicity and reduced fertility rate in affected females. Supported by NIH P01AI003961, R01AT006888, R01MH094755, P20GM103641, R01AI129788 and R01AI123947 on PN and MN.

Phthalates are a class of chemicals that cause developmental and reproductive toxicity. They are widely used to plasticize polyvinyl chloride for use in medical devices, industrial, and commercial products. Humans are frequently exposed to phthalates which are not completely bound to PVC. Despite their known adverse effects on male reproductive development, there are significant gaps in understanding of the mechanisms of phthalate toxicity. Retinoic acid signaling is involved in both spermatogenesis and fetal gonad development. There is evidence that phthalates disrupt retinoic acid signaling. However, the contribution of this interaction to phthalate toxicity is unclear. We hypothesized that mono-(2-ethylhexyl) phthalate (MEHP) would enhance the toxicity of all-trans retinoic acid (ATRA) during mouse fetal testis development. To test this hypothesis, gestation day 14 C57BL/6 mouse testes were isolated and cultured on media containing MEHP (10^{-6}, 10^{-5}, or 10^{-6} M), ATRA (10^{-8}, 10^{-7}, or 10^{-6} M), a co-exposure consisting of 10^{-6} M ATRA with the full concentration range of MEHP, or vehicle control (1:4000 DMSO). After one day of exposure, samples were snap-frozen to isolate RNA for global transcriptome analysis. After three days of culture, tissues were fixed for histological analysis and immunofluorescent labeling of SOX9 (Sertoli cells), and FOXL2 (aberrant granulosa cell marker). Exposure to ATRA disrupted seminiferous cord morphology and induced FOXL2 expression. Co-exposure to MEHP and ATRA increased the number of FOXL2-positive cells and reduced seminiferous cord number, relative to ATRA alone. The total number of significantly differentially expressed genes following exposure to ATRA and MEHP was 510 and 134, respectively, 70 of which were common to both MEHP and ATRA. This supports our hypothesis that MEHP modulates retinoic acid signaling in the mouse fetal testis. RNA-seq analysis found that MEHP, ATRA, and the co-exposure all altered the expression of homeobox genes, including Otx2, Lhx1, and Pax2, which may play a role in spatial patterning. MEHP and ATRA also significantly downregulated the expression of Pdgfa, a key signaling factor expressed by Sertoli cells during cord development. Co-exposure with MEHP enhanced Pdgfa down-regulation by ATRA, which may explain the disruption of cord morphogenesis.

Phthalates interact with Retinoic Acid Signaling Alters Fetal Mouse Testicular Somatic Cell Differentiation and Spatial Patterning

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Consumer products brands are requiring increased transparency and higher standards for safer chemistry from suppliers throughout their supply chains. Historically consumer product chemical safety has focused on regulatory compliance. Compliance is typically achieved through Safety Data Sheet (SDS) checklist. Authorities are not in consensus on how crucial CFFD is to fully scoring and screening formulations to eliminate chemicals of high concern and select safer chemicals. Within the case studies, specific examples of chemicals with vCHAs revealing chemicals of high concern will be demonstrated along with how ALC and SDS disclosure can miss highly problematic chemicals being reported upstream the supply chain. Ultimately, brands, retailers, suppliers, workers, and consumers can benefit from this work which creates a system for continuous improvement in safer and sustainable chemistry.

Impaired Hair Care Product Exposure on Hair Follicle Health

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A human hair follicle is comprised of a number of specialized cells and is located in the epidermis of the skin. While it is responsible for the health and growth of the hair, there is no standardized assay to evaluate the impact of exogenous agents on hair follicle health. Recent reports of hair loss due to use of various hair care products have prompted interest in the potential impact of normal product usage on hair follicles. Due to the lack of validated systems, two novel ex vivo culture systems were applied to evaluate hair follicle health: the human hair follicle dermal papilla cell (HFDPc) culture system and the HairSkin model. Dermal papilla cells (DPCs), often described as stem cell-like cells, are specialized mesenchymal cells located inside human hair follicles. DPCs play a critical role in the development and growth of both the hair and entire hair follicle. Primary HFDPCs isolated from a female donor were cultured and treated with commercial hair care products. Repeated exposure to a physiologically relevant concentration of the product did not lead to significantly increased cytotoxicity and change in cell morphology that was observed when treated with 0.02% sodium dodecyl sulfate (SDS). The HairSkin model allows for scalp skin samples containing hair follicles from cosmetic procedures to be cultured ex vivo. These cultures are used to evaluate the potential impact on hair follicle health following exposure to test articles. Scalp skin samples obtained from a female donor were cultured and treated with a commercial hair care product. Treatment with neat commercial hair care product did not result in changes to structure integrity, cell proliferation, and cell apoptosis. While the systems evaluated are exploratory, they provide qualitative information on potential effects resulting from exposure to commercial hair care products tested. Further development and validation may prove advantageous to advancing consumer safety. Overall, these findings suggest that exposure to commercial hair care products evaluated at the tested concentrations does not adversely impact overall health of hair follicles.

Full Formulation Disclosures (CFFD). CFFD ensures chemical disclosure for all intentional ingredients and down to 100 ppm (or below) for impurities and specific restricted chemicals. Full disclosure is obtained from multi-tier supply networks and protects trade secrets for each participant in the process being hedged. Over the last 12 years the Scivera team has completed over 4,000 verified chemical hazard assessments (vCHAs) for chemicals in consumer products, and hundreds of CFFD reviews of chemical products with chemical screening and scoring. In this work we identified significant challenges including obtaining CFFD within multi-tier supply chains requiring security of confidential business information (CBI). Beyond the observed challenges, the CFFD process reveals chemicals present in formulations as intentional ingredients or impurities that were previously missed in safety / sustainability evaluations and significantly impact the human or environmental impact of the formulation overall. Case studies will be presented on best practices to obtain CFFD while maintaining CBI and illustrate how crucial CFFD is to fully scoring and screening formulations to eliminate chemicals of high concern and select safer chemicals. Within the case studies, specific examples of chemicals with vCHAs revealing chemicals of high concern will be demonstrated along with how ALC and SDS disclosure can miss highly problematic chemicals being reported upstream the supply chain. Ultimately, brands, retailers, suppliers, workers, and consumers can benefit from this work which creates a system for continuous improvement in safer and sustainable chemistry.

2748 Chemical Characterization of Contemporary Shisha Tobaccos

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Numerous journal articles have addressed the adverse health effects associated with waterpipe (hookah) smoking of shisha tobaccos. Most assumed that all shisha tobaccos and waterpipe use were equivalent. This is not the case as shisha tobaccos differ in composition as do the conditions of waterpipe use, such as heat source and tobacco temperature during use. A recent report provided data on the amounts of glycercin (G), propylene glycol (PG), and water (W) in commercial shisha tobaccos. The combined amounts of G, PG, and W ranged from a high of over 50% of shisha weight to a low of 15%. This volatile content is important as reported shisha temperatures during use were from 51 to 73%, sugars ranged from 7 to 26%, and levels of heavy metals and toxicants in emissions from heated shisha products. Consequently, we had 16 heated hookahs are used. However, apparently no reports to date have provided other relevant information on types of tobacco used and the specific sugars found in the shishas, as both can affect the production of CO and other toxins in emissions from heated shisha products. Consequently, we had 16 consumer products that we evaluated. Treatment with neat commercial hair care product did not result in changes to structure integrity, cell proliferation, and cell apoptosis. While the systems evaluated are exploratory, they provide qualitative information on potential effects resulting from exposure to commercial hair care products tested. Further development and validation may prove advantageous to advancing consumer safety. Overall, these findings suggest that exposure to commercial hair care products evaluated at the tested concentrations does not adversely impact overall health of hair follicles.

2745 Applying Chemical Hazard Assessment in the Consumer Products Supply Chain: Challenges and Best Practices to Ensure Safer Chemistry Solutions

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Consumer products brands are requiring increased transparency and higher standards for safer chemistry from suppliers throughout their supply chains.

2746 Impact of Personal Care Product Exposure on Hair Follicle Health

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Scivera’s toxicology team works with brands, retailers, and their suppliers in many product categories including footwear and apparel, electronics, beauty, amongst others to screen and score chemistries through Confidential Full Formulation Disclosures (CFFD). CFFD ensures chemical disclosure for all intentional ingredients and down to 100 ppm (or below) for impurities and specific restricted chemistries. Full disclosure is obtained from multi-tier supply networks and protects trade secrets for each participant in the process being hedged. Over the last 12 years the Scivera team has completed over 4,000 verified chemical hazard assessments (vCHAs) for chemicals in consumer products, and hundreds of CFFD reviews of chemical products with chemical screening and scoring. In this work we identified significant challenges including obtaining CFFD within multi-tier supply chains requiring security of confidential business information (CBI). Beyond the observed challenges, the CFFD process reveals chemicals present in formulations as intentional ingredients or impurities that were previously missed in safety / sustainability evaluations and significantly impact the human or environmental impact of the formulation overall. Case studies will be presented on best practices to obtain CFFD while maintaining CBI and illustrate how crucial CFFD is to fully scoring and screening formulations to eliminate chemicals of high concern and select safer chemicals. Within the case studies, specific examples of chemicals with vCHAs revealing chemicals of high concern will be demonstrated along with how ALC and SDS disclosure can miss highly problematic chemicals being reported upstream the supply chain. Ultimately, brands, retailers, suppliers, workers, and consumers can benefit from this work which creates a system for continuous improvement in safer and sustainable chemistry.
**2749 Guidance on Dose-Setting in Repeated-Dose Toxicity Studies: Outcome of an ECETOC Task Force**

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The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) has established a Task Force with the objective to provide guidance on dose-setting to support appropriate design and interpretation of toxicity studies. Recent regulatory opinions mainly in the EU, and driven by classification needs, have suggested that there should be a systematic move to increase the dose levels used in repeat dose toxicity studies, combined with a resistance to the use of kinetics to inform dose level selection. Beside animal welfare consideration, inappropriate dose setting hinders our ability to correctly estimate human risk while overestimating hazard, identifying the urgent need for science-based guidance. In the development of the guidance, we propose practices that reflect the current state of the science, considering the following elements. A) Discussion of the dilemma of studies expected to fulfill a dual purpose of providing information for risk assessment as well as for hazard characterization, indicated that human-relevant and exposure-led information should be preferentially used in dose selection where appropriate. B) The critical review of the basis for using a Maximally Tolerated Dose led to a consensus that there is no direct link between increasing doses beyond this value and better protection of human health, neither for serving classification needs or for risk assessment/risk benefit analysis. C) Existing practices in dose setting across industrial sectors and regulations have been reviewed, including the value of range-finding in vivo studies. The review included dose selection parameters indicated in current (primarily OECD) test guidelines, some improved assessment of maternal toxicity in reproductive studies and preparation of sector specific recommendations (including pharmaceuticals, chemicals, agrochemicals, biocide, food). D) Guidance for use of toxicokinetic and pharmacodynamic evidence in dose setting was prepared reviewing approaches and examples, confirming that testing in non-linear kinetic ranges correspond to exceedance of Maximum Tolerated Doses.

**2750 Application of the Capillary Aerosol Generator (CAG) to Generate Aerosols for E-liquid Preclinical Inhalation Studies**

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Electronic nicotine delivery systems (ENDS) have gained popularity with various types of products in the commercial marketplace. While most ingredients used in e-liquids are generally recognized as safe for oral consumption, there is limited available information to evaluate their inhalation toxicity. In this study, we evaluated the suitability of a capillary aerosol generator (CAG) which can generate condensation aerosol from e-liquids for preclinical inhalation safety studies. A base formulation (BF) containing propylene glycol (PG), glycerin, water and nicotine and a flavored formulation (Mix) containing the base and flavor ingredients were used to generate aerosol atmospheres for nose-only inhalation exposures. Aerosol samples were collected with Cambridge filter pads and/or impingers at the nose ports and characterized for aerosol mass, major ingredients (PG, glycerin, and nicotine), selected carbonyls (e.g., acetaldehyde, acrolein, and formaldehyde), particle size distribution (by cascade impaction), and exposure port uniformity (gravimetric) during exposures. Results were compared to a published inhalation study (Lee et al. 2018) in which commercial cig-a-like ENDS products filled with the same formulations (BF and Mix) were puffed (55 ml puff volume, 5-second puff duration, and 30-second inter-puff interval) using rotary smoking machines to generate aerosol exposure atmosphere for mice. The CAG consistently delivered aerosols for repeat dose studies with an aerodynamic diameter of 1.0 - 1.3 μm and the geometric standard deviation of 1.4 - 1.7 with aerosol mass concentrations ranging from ~250 μg/L to ~5500 μg/L at the nose port. The CAG aerosols contained similar composition of PG, glycerin and nicotine at the nose ports as the ENDS product aerosols from the same formulation. When detected above the limit of quantitation, the levels of formaldehyde and acrolein were slightly higher while acetaldehyde was slightly lower in the CAG aerosol compared to the ENDS product aerosol, but overall the levels of the carbonyls in both the CAG and product aerosols were of a similar order of magnitude. In summary, the results support that the CAG is a pragmatic option to generate comparable and controllable e-vapor aerosols to study preclinical inhalation toxicity of ENDS ingredients.

**2751 Unique Challenges in Generating Microparticle Particles for Toxicological Research**


Microparticle (MP), which can be defined as synthetic water-insoluble solid particles with size ranging from 1 to 5000 μm, pose a public health concern. Numerous information gaps exist in order to complete human health and environmental risk assessments. Lack of relevant test materials is a unique challenge limiting progress in toxicology studies; therefore, Dow R&D and Toxicology have partnered to develop well characterized MPs. We synthesized an aequous dispersion of ethylene acrylic acid copolymer MP for use in aquatic Daphnia magna experiments. This spherical MP was stable in an aquatic dispersion, had an average particle diameter of 103 nm, a density of 1.2 g/cm3, and contained 91% of the absolute zeta potentials were 17.1-18.4, indicating moderate colloid stability. Limited agglomeration was observed during our D. magna experiments. For future mammalian and eco-toxicology studies, BLUZWAVE™ technology is being used to synthesize polyethylene MP targeting prioritized size ranges (i.e., < 10 μm as these have the greatest aerosol traverse biological membranes) with labeling to allow experimental tracking. This technology uses a high internal phase emulsion process with a polymer melt phase, dispersant and water in a primary mixer to create polymer dispersions with controlled particle size ranges. To date, experiments have shown that differing dispersants are necessary to create large (μm) and small (nm) particle sizes, making this variable difficult to control across experiments. Furthermore, particles <5 μm have high surface area, allowing interfacial energy to dominate and causing particles to agglomerate, which can be problematic without addition of surfactants. Furthermore, exposure to low density MPs may be challenging in in vitro systems. Following polyethylene MP sample synthesis, characterization of particle size, shape, concentration and composition, surface reactivity/agglomeration potential, and label stability is conducted. To examine intestinal uptake of MP samples, air-liquid interface models may allow exposure to low-density samples in minimal media. To date, this work has demonstrated that there are fundamental challenges in the development of MP test materials, requiring unique particle chemistry expertise and manipulation of variables atypical to the field of toxicology.
Plant extracts are increasingly used in cosmetics, mainly as bioactive ingredients. The growing interest in employing bioactive ingredients propels the demand for evaluation of differentiated plant extracts. We have prepared two marine-sourced plant extracts (Macrocytis pyrifera and Laminaria saccharina) using an electrokinetic approach that preserves the inherent osmotic pressure of the plant cell juice and uses it as a separation medium followed by mechanical and gentle separations without using hazardous solvents. Their application in personal care products is novel and requires evaluation for their safety use by consumers. Our safety assessment approach involved: (a) elemental / composition analysis, (b) exposure assessment, (c) safety thresholds or reference doses review, (d) in vitro toxicity and skin compatibility study, and (e) risk characterization. Based on the elemental analysis, iodine in extracts is identified as a key substance in consumer health risk characterization. While an effort to reduce the total iodine levels to be less than 20 ppm is in progress, the estimated dermal exposure (less than 0.01 mcg / sq cm) to iodine present in the current extracts (less than 200 ppm) is significantly lower than the levels anticipated to be present in human skin when the extracts are being used in personal care products up to 5%. The systemic exposure (less than 1.8 mcg / day) to iodine present in the extracts to be used in personal care products is also lower than the total safety threshold limit (300 mcg / day Minimal Risk Level) or Recommended Daily Allowances for iodine (150 mcg / day). The safety of both extracts is also supported with in vitro toxicological and skin compatibility studies: (a) in vitro skin irritation using the Ashland Epidermis Equivalent (not irritating), (b) Epiderm™ eye irritation (not irritating), (c) Ames assay with Salmonella typhimurium and Escherichia coli strains (not mutagenic), (d) 3T3 neutral red uptake phototoxicity assay (not cytotoxic/phototoxic), (e) 48-hr human patch testing (not irritating), and (f) human repeat insult patch testing (not irritating or sensitizing). Based on this assessment, the use of both extracts as raw ingredients of personal care products up to 5% is judged to be safe under normal use and foreseeable misuse conditions.

Human iPSC-derived cardiomyocytes (iPSC-CM) are a well-established model system to leverage public and commercial resources to improve in silico chemical safety assessment. Human iPSC-derived cardiomyocytes (iPSC-CM) are a well-established model system to leverage public and commercial resources to improve in silico chemical safety assessment. The use of non-animal testing-based alternative methods in chemical safety assessment has made much progress. While the replacement of animals requires further development of alternative methods for repeated-dose, reproductive and developmental toxicity and understanding of toxicokinetics, the knowledge developed from regulatory programs such as REACH is actively being leveraged. The formalized methods of quantitative structure-activity-relationships (QSAR), grouping and read-across are also applicable to sectors beyond REACH. Experimental toxicity data for cosmetics ingredients, food additives, agrochemicals and their metabolites, and impurities can be included to enhance the applicability domain of the knowledgebase. COSMOS Next Generation (https://ng.cosmosdb.eu) provides relevant assessment data, whilst continuing to host data from EFSA, ECHA, and US FDA. COSMOS NG is a gateway for the commercial ChemTunes.ToxGPS package to publicly share data and new methodologies across industry and regulatory agencies. Due to this flexibility, Cosmetics Europe has also joined the commercial-public resource sharing effort, with the goal of contributing to a chemoinformatics platform to aid toxicity predictions of cosmetics-relevant chemicals. The system has captured workflows based on CE case studies (e.g., paraben, caffeine) for TTC and read-across utilizing in silico, biokinetics, and metabolism results, as well as high throughput and high content mode-of-action and toxicity studies. The aspiration is to represent an ab initio workflow based on case study experiences. Collaboration between public and commercial entities will expand the knowledge domain and increase reliability. This work is funded through the Long Range Science Strategy (LRSS), a program of Cosmetics Europe, with participation of industrial members.
values were predicted by reverse dosimetry simulation using a PBPK model. The co-culture 3D cardiac microtissues retain their total cells the increased positive inotropic response to 2-fold (100% change) at NICEATM, Research Triangle Park, NC; and 3US EPA, Washington, DC.

Prior to registration and sale, agrochemicals must be characterized for potential risks associated with exposure through all possible routes, including the dermal route. Dermal toxicity is primarily driven by the ability of a substance to penetrate the skin and subsequently pass into the systemic circulation. An estimate of dermal absorption is possible using the “triple pack”, a study design that combines results from in vivo rat, in vitro rat, and in vitro human studies to calculate an estimated human dermal absorption factor (DAF). To assess the feasibility of deriving a DAF using only in vitro data, we conducted a retrospective evaluation to compare the DAF derived from each of the three methods (rat in vivo, rat in vitro, and human in vitro). Additionally, the DAF derived from the human in vitro study was compared to the DAF generated from the triple pack approach. In over 70% of the 30 agrochemicals evaluated, the ratio of in vitro to in vivo absorbance in rat skin was greater than one, indicating that the in vitro rat method generated a similar or higher DAF value than the in vivo method. Consistent with other studies that have demonstrated greater permeability of rat skin compared to human skin, absorption through in vitro human skin was similar to or less than that observed in rat skin for all 30 agrochemical formulations evaluated. For most of the chemicals evaluated, the human in vitro method provided a similar or higher estimate of dermal absorption than the triple pack approach. In cases where the human in vitro method provided a lower DAF value, most were within less than 1% to 4% of the values obtained from the triple pack approach, indicating the human in vitro and triple pack approach provided similar estimates of dermal penetration in these cases. This project was funded with federal funds from the NIH under Contract No. HHSN27220150010C.

In vitro-to-in vivo extrapolation could bridge between in vitro toxicity and an in vivo dose that causes toxic effects therefore enabling rapid and effective safety evaluation. Here, a combined in vitro cytotoxicity and in silico reverse dosimetry approach was adopted to evaluate the potential human liver toxicity of 16 anthraquinones and derivatives. First, cytokotoxicity (IC_{50}) in two human liver cell lines (HepG2/Cl3A and HuH-7) was measured under two conditions (single and repeated dosing, 72 h). Second, toxic doses (D_{tox}) required to yield plasma steady-state concentrations (C_{ss}) equal to in vitro EC_{50} values were predicted by reverse dosimetry simulation using a PBPK model. Finally, D_{tox} was compared to literature-derived estimated daily intake (EDI) of anthraquinones to assess liver safety among the 16 anthraquinones. Rhein was identified as a potential hepatotoxican due to a combination of cytotoxicity, plasma concentration, and daily intake level. These in vitro and in silico findings provide preliminary data and guidance for further animal and clinical studies to confirm liver toxicity of anthraquinones.

The USEPA intent to eliminate reliance on vertebrate tests from 2035 challenges the research-led Crop Protection (CP) companies to develop new approaches in order to meet the regulatory requirements for new Active Ingredients (AI), as no guidance currently exists to meet the EPA’s goal whilst ensuring no unreasonable risk to human health and the environment. To determine the scope and direction of a project, Syngenta and USEPA OPP senior leadership and scientific staff held a program formulation discussion resulting in a draft problem statement: “Establish a scientifically sound strategy that applies appropriate and flexible exposure and effects characterization without chemical-specific vertebrate tests to address the risk assessment needs that provides confidence in regulatory decisions”. To resolve this problem, we set to partner with USEPA on a project where data generated according to the current data requirements and test guidelines, as well as the new approaches can be submitted and reviewed in parallel for the purpose of new AI registration.

The project objectives are to create a worked example applying existing conceptual frameworks based on modern scientific approaches to identify and characterize the dose-range over which potential adverse effects may occur relative to the anticipated exposure from proposed uses of a new AI, determine whether these frameworks meet the USEPA’s risk assessment needs for human health and vertebrate ecotoxicology, and to identify where further development of new approaches for complex specific data generation would be required. To maximise the reuse of existing data, we selected a new herbicidal AI with an established mode of action having many exemplars for which the toxicology and ecotoxicology is well characterized. We demonstrated that these data can be curated and analysed to provide an appropriate human health and vertebrate ecological hazard characterisation for the purpose of risk assessment of such new AI. We further explored its possible extension to CP chemicals with less extensive existing exemplar datasets and highlighted the identified uncertainties and gaps that will require additional new approaches.

The in vivo rabbit test is the benchmark against which new approach methodologies for skin irritation are usually compared. In vitro methods are accepted as partial replacements for the in vivo tests because they can distinguish irritant from non-irritant chemicals (as defined by the rabbit test). However, because none can classify substances as moderate and mild irritants, there are currently no in vitro methods that are considered full replacements for the in vivo assay. A limiting factor in identifying a complete replacement could be the variability inherent to the subjective scoring of erythema and edema responses in the rabbit test. This is particularly relevant for mild and moderate irritants, where interindividual differences in scoring are most likely to occur.

To better characterize the reproducibility of the in vivo assay, we assessed variability in animal study results from substances tested multiple times. We compiled and curated 2634 test records, representing 990 unique mono-constituent substances, each tested at least twice. Methodological deviations from guidelines were noted, and multiple data sets with differing tolerances for such deviations were created. Where possible, primary dermal irritation indices were estimated from the available data and used to classify chemicals according to the U.S. Environmental Protection Agency (EPA) skin irritation classification criteria. Globally Harmonized System (GHS) hazard classifications were also extracted from study reports when available. Probabilities were used to evaluate the reproducibility of the in vivo method in identification of EPA or GHS hazard categories. Chemicals classified as moderate irritants at least once were classified as mild irritants or non-irritants at least 40% of the time when tested repeatedly. Variability was greatest between mild and moderate irritants, which both had less than a 50% likelihood of being replicated. Increased reproducibility for the EPA and GHS systems was observed when a binary categorization was compared between corrosive/moderate irritants and mild/non-irritants. This analysis indicates that variability present in the rabbit skin irritation test should be considered when evaluating the performance of nonanimal alternative methods as potential replacements. This project was funded in whole or in part with federal funds from the NIH under Contract No. HHSN27220150010C.
Although 80,000 chemicals are used in commerce, 98% have little-to-no toxicity information. To address this data gap, the Toxicology Testing in the 21st Century (Tox21) program used a 1536-well plate format to conduct quantitative high-throughput screening assays (qHTS) to gain toxicity information on a 10,000 chemical library primarily composed of chemicals of environmental concern. Due to technical limitations in the initial screening program, standard methods for supplying exogenous metabolic activation could not be used in these systems. This study used the Invitrogen CellSensor p353E beta-lactamase reporter assay to evaluate whether adding a metabolic capability could identify additional compounds that induce p35 signaling after biotransformation. p35 is induced in response to DNA damage and other cellular stressors. Metabolic activation was supplied by either induced rat liver microsomes (RLM) or human liver microsomes (HLM). The 10K library was screened using the p353E assay without microsomes, with RLM, or with HLM. Results showed that 276 compounds were active in any one of these three experimental conditions. Of these 276 chemicals, 48 compounds (e.g., phorate and chlorpyrifos) were more potent in the p353E + RLM assay, and 8 compounds (e.g., methamidophos) were more potent in the p353E + HLM assay compared to p353E without microsomes. To test whether these changes in potency were truly due to metabolism, we further tested these compounds using RLM or HLM that were heat inactivated, treated with a CYP inhibitor, or applied to the test system in the absence of NADPH. Most of the 56 compounds became less potent under these treatment conditions, confirming that the metabolic transformation was responsible for the increased potency. In future studies, we seek to confirm that these compounds undergo metabolic activation by measuring changes in the level of the parent compound in primary hepatocytes and screening the compounds using in silico metabolic prediction software (e.g., ADMET). Taken together, this approach shows promise for identifying chemicals in high throughput screens that require metabolism to induce a biological response.

2762 In Vitro, In Chemico, In Silico, or In Vivo for Evaluation of Airborne Chlorothalonil Aerosols to Estimate a Safe Level of Exposure for Workers

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Movia et al. (2020) published a review (https://doi.org/10.3389/fbioe.2020.00549) regarding in vitro alternatives to possibly replace in vivo acute inhalation studies in animal models. Among 12 suggestions, four specific requirements were included regarding extrapolations to humans from in vitro assays. The four major items cited to extrapolate from an in vitro assay to human respiratory tract system were: 1. Comparison to tissue in humans. 2. Comparison to known human-relevant exposure scenarios (exposure methodology). 3. Human-relevant endpoint definition and 4. Human-relevance. They also quoted two reports from (EPA 2019, 2020) comparing an in vitro assay to replace current acute inhalation animal toxicity protocols to evaluate safety for chlorothalonil and a third report (EPA 2019), providing the evaluation of the proposed in vitro assay. This chemical is a solid, and worker’s exposure will be by inhalation of aerosols. It is known that it can induce a wide variety of irritation effects (sensory and inflammatory) on the respiratory system, as well as severe eye irritations (EPA 2019). Safe levels of exposure for the different types of exposure in workers are needed. After reviewing the above, the data suggest using in chemico from Tarantino and Sass, 1974, (https://doi.org/10.1006/0041-008X(74)90030-1) instead of in vitro for this chemical. One bioassay meeting the four human relevant requirements cited, is an in vivo assay, the RDS0 mouse bioassay for sensory irritation (Vijayaraghavan et al. 1994, (https://doi.org/10.1007/s00240050101). However, trigeminal nerve endings are similar enough in humans and mice to satisfy item 1 above and the other items are also satisfied. For items 1, 2, 3 and 4, the data have not yet been presented for relevance to humans, except for the mouse sensory irritation in vivo RDS0 described by Schaper, 1993 (DOI: 10.1080/1529866931355017). Taking the example of the in vivo RDS0 bioassay, the steps required to replace it by in chemico, in silico (QSAR) or in vitro will be presented, specifically to estimate a safe level of exposure for humans to chlorothalonil, while meeting the four relevant human items. A list of references cited above and in the poster, will be available upon request at rdso@pitt.edu.

2763 Quantifying the DARTable Genome for Prediction of Teratogenic Doses: A Case Study Using Retinoic Acid Pathway-Induced Developmental Toxicity


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The conservation of developmental processes permits the application of a quantitative adverse outcome pathway (AOP) model to predict threshold doses resulting in teratogenic effects. The goal of the HESI DARTable Genome Working Group is to build a comprehensive framework of molecular initiating events (MIEs) and key event biomarkers for teratogenicity, which could eventually enable toxicologists to profile potential teratogenicity to assess risk of chemicals, aiding in prioritization of chemicals for further testing, and ultimately reduce animal testing for some molecular entities as the requisite regulatory guidelines incorporate evolving science. Here, we used the retinoic acid (RA) pathway as a case study and explored the application of an AOP model that investigates the relationship between putative molecular initiating event (MIE), retinoic acid receptor (RAR) potency, and the quantitative threshold of maternal systemic exposure necessary to produce a teratogenic outcome. Utilizing publicly accessible data, we curated RA-induced kinetic, and toxicity information for 20 chemicals known to act on the RA pathway. Potency data for all RAR isoforms was available for 12 compounds, pharmacokinetic data for 5 compounds, and toxicology information for 9. Using only those compounds with all three data types, we identified a path- way (e.g., maternal exposure, RAR potency, and teratogenicity) for 3 compounds in the rat and 2 in the rabbit. A maternal blood exposure (AUC24) to RAR-potency ratio of > 2 for either RARα or RARγ was associated with a lowest observed effect level (LOEL) in the rat, while a ratio < 1.3 was associated with a no observed effect level (NOEL). The NOEL and NOEL ratios in the rabbit were 0.4 and 0.13, respectively. Ratios calculated relative to RARβ did not correlate with LOELs, potentially due to the known ability of RARβ to be complemented by the other RAR subtypes. Extending this analysis to test the predictivity of these ratios for other retinoids requires data that may currently be unpublished. Further, the discordant results between species suggests that further work is necessary to understand quantitative species differences in the RAR/RA pathways, transplacental transfer, teratogenicity, and maternal toxicity. This abstract does not reflect US EPA or FDA policy.

2764 Prediction of Inter-ethnic and Inter-individual Variations in the Cardiotoxicity of R- and S-Methadone by Integrating Monte Carlo Simulations and Physiological-Based Kinetic Modeling-Based Reverse Dosimetry

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Within the framework of developing new approach methodologies (NAM) to predict human toxicity, the present study aims to demonstrate an in vitro-in silico approach that predicts the effect of inter-ethnic and inter-individual kinetic variations for the sensitivity towards the cardiotoxicity of R- and S-methadone in the Caucasian and Chinese population. The inter-ethnic and inter-individual variations in both populations. Results show that the calculated relative to RARβ did not correlate with LOELs, potentially due to the known ability of RARβ to be complemented by the other RAR subtypes. Extending this analysis to test the predictivity of these ratios for other retinoids requires data that may currently be unpublished. Further, the discordant results between species suggests that further work is necessary to understand quantitative species differences in the RAR/RA pathways, transplacental transfer, teratogenicity, and maternal toxicity. This abstract does not reflect US EPA or FDA policy.

2761 Profiling Tox21 Compounds That Activate p53 Signaling after Metabolic Activation

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Aerosols to Estimate a Safe Level of Exposure For Workers
NexGen Risk Assessment (NGRA) is an exposure-led, hypothesis-driven approach integrating new approach methodologies (NAMs) to ensure safety without generating animal data. We have developed an NGRA framework for Skin Allergy that is based upon ICC principles (Dent et al., 2018[WC1]) and aligns with the Cosmetics Europe Skin Allergy NGRA framework (Gilmour et al., 2020). This is applied to a hypothetical skin allergy assessment of a consumer product at two exposures - 0.1% coumarin in a face cream and 1% in a deodorant. This follows a parallel publication, Baltazar et al., 2020, on the systemic toxicity NGRA for coumarin. For the purposes of the case study, animal data, clinical data and read-across were not used, and the use of dermal sensitisation threshold models is not aimed at estimating estimates for coumarin from the two hypothetical products were determined (SCCS, 2018). Expert structural assessment and in silico chemistry predictions (ToxTree, OECD Toolbox, DEREK) gave alerts for skin sensitisation. Both direct and indirect (pro-hapten) mechanisms were indicated. Data generation for coumarin was required to serve as inputs to the Skin Allergy Risk Assessment (SARA) Model (Reynolds et al., in draft). The SARA Model is a Bayesian approach which allows prediction of a human relevant point of departure (PoD) (the HIRIPT dose with a 1% chance of sensitisation or ED1% (µg cm⁻²)) based upon any combination of HiRPI, historical LLNA, DRA, KeratinoSens, h-Clat or u-Sens data. Here we ran the generated in chemico and in vitro data only to infer the ED1% for coumarin. The PoD for coumarin benchmarked with weak sensitisers within the database. In addition to the potency prediction, by incorporating benchmark exposure information, the SARA Model can be used to calculate a measure of sensitisation risk for a consumer relevant exposure with quantified uncertainty. The model predicts with high probability that coumarin is low risk at 0.1% in a face cream, however predicts 1% coumarin in a deodorant as high risk. Additional data on coumarin metabolism was generated, in human liver S9 incubations and NativeSkin® models, to support SARA outputs. However, the results of these studies did not alter the respective low and high risk outcomes of the SARA Model was investigated by using specific inhibitors in a weight of evidence. This case study provides an example of the value that integrating exposure science with computational modelling and in vitro bioactivity data has for non-animal NGRA.
Transcriptomic Model to Predict General Toxicity and Endocrine Disruption

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In 2018 the Government of Canada began researching the potential of the zebrafish (ZF) embryo and larva as whole organism models to 1) evaluate endocrine disruption beyond developmental effects and 2) as an alternative to the 28-day rodent assay for general toxicity assessments, thereby facilitating the 3Rs of reduction, refinement and replacement of animals in toxicity testing. Key to this research are 1) incorporation of behavioral and transcriptomic platforms and endocrine biomarkers of general toxicity and 2) incorporation of collaborative validation to create a ZF model as a globally harmonized regulatory tool for chemical risk assessment. Using NRC Canada ZF toxicity tests, the fish embryo toxicity (FET) assay and the general and behavioural toxicity (GBT) assay, 3 replicates of 12 embryos were treated with 10-4 to 100 μM of 6-120 hpf in the FET and for 72-120 hpf in the GBT. Single larvae (GBT) or chlorinated embryos (FET) were treated in individual wells. Selection of 7 concentrations/test compound was based on the MTC determined in dose range finding tests. Phenotypic toxicity (EC20) was profiled using 20 markers (e.g., heart, tail, heart beat) at 72 and 120 hpf in the FET and 96 and 120 hpf in the GBT. Behavior was evaluated for distance travelled and startle response at 120 hpf. Larval tissue was collected for RNA sequencing to determine differentiable gene expression. In collaboration with the NTP (SEAZIT initiative), 20 test compounds (Aldicarb, Amoxicillin, Benzophenone, Bisphenol A, Bisphenol S, Dechlorane Plus, 3,4-Dichloroaniline, Raloxifene HCl, Piriproxfen, Pyrene, Propofol, Ritonavir, Tramadol, Tretinoin, Tetramethoxybenzophenone, Tridecyl Propionate, Thienobenzodiazole, Tricresyl Phosphate, Triphenyl Phosphate, Tris(dichloro-isopropyl) phosphate, Valproic Acid) were selected. The EC20 for the most prominent phenotype observed varied with substance, and between assay (FET, GBT) and time points. The behavioural response varied with substance, and general toxicity as potentially more sensitive than rodent assays. And life-stage specific sensitivity that support the utility of ZF models with their biological similarity, and thus the validity of the read across among the group. Supported by Cosmetics Europe (https://www.lisscosmeticeurope.eu).

Use of Transcriptomics to Identify Cosmetic Ingredient Biological Activity: Caffeine and Its Metabolites

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The objective of this work was to use transcriptional signatures to assess the biological activity of caffeine and its main metabolites (theophylline, theobromine and paraxanthine) to define their biological similarity and with that, substantiate the validity of a read across approach usable in risk assessment. The comprehensive transcriptional response of MCF7, A549, HepG2 cells and cardiomyocytes was evaluated (TempoQ-Seq, BioSpyder) after exposure to vehicle-control and 1-3 μM each of methanxanthines at 3 non-cytotoxic concentrations (50, 500 and 1000 M), for 6h. Differentially expressed genes (FDR ≥ 0.05, and fold change ≥ 1.2x) were identified. The transcriptional profile elicited of all four compounds had a high similarity across the cell types. The most robust response was observed in the cardiomyocytes with the highest transcriptional profile similarity between caffeine and theophylline. In these cells, we identified 638 common genes whose expression is modified by these 2 methylxanthines in a significant manner and in the same direction. The transcriptional profile of the methylxanthines is similar to the one elicited by sirolimus, wortmannin, and LY-294002 (among others), chemicals known as mTORC1 signaling, cell cycle related targets of E2F transcription factor, G2/M checkpoint and oxidative phosphorylation. This was indicative of their biological similarity, and thus the validity of the read across among the group. Supported by Cosmetics Europe (https://www.lisscosmeticeurope.eu).

Case Study on the Use of New Approach Methods to Refine and Strengthen the Toxicological Structure-Activity Relationship Read-Across for Branched-Alkylcarboxylic Acids

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This work demonstrates the value added by new approach methods (NAMs) in the toxicological read-across for branched alkyl alcohols/sulfates and corresponding acids. Literature indicates that two branched chain alkyl (C5-C6) carboxylic acids with specific structural features have developmental and reproductive toxicity (DART), 2-ethyl hexanoic acid (EHA), valproic acid (VPA). The lack of DART data on longer branched chain structures (> or ≥C12) makes assessing the DART endpoint challenging and can potentially lead to the whole ‘class’ being as assumed to be DART toxicants. There is a need to distinguish the ‘class’ based on length of alkyl chain and branched side chain. Chemical similarity evaluations, in silico information, toxicogenomics, in vitro toxicokinetic (TK) data and physiologically based pharmacokinetic (PBPK) models were used to provide evidence of the chemical and biological (dis) similarity of branched-alkylcarboxylic acids (i.e. C5 - C8 vs C12 and longer). Data indicates a similarity in physico-chemical properties of the branched chain alkyl carboxylic acids and an association of decreased DART effects as the branched chain increases. A transcriptomics approach was used to inform on the biological activity and similarity of the compounds. Toxicogenomic data indicate that alkyl substituents (C2 or ≥C2) at the α position of the carboxylic acid are associated with DART effects (VPA, EHA) and these chemicals elicit a similar transcriptional profile. As the branched chain length increases, the DART effects appear to decrease and their transcriptional profile is different than the one from VPA. Methyl branched alkyl acids have weak DART effects as compared to VPA. An in vitro TK data show similarity across compounds for some parameters (caco-2 permeability) and dissimilarity in other parameters (metabolism). An in vitro TK data was used in PBPK models to illustrate the impact on intestinal absorption. Overall, this work expands our understanding for branched alkyl acids and demonstrates how NAMs can improve the toxicological RAX. This work was supported by Cefic.
adapted to be used for screening. Also, the newer RNA-seq technologies are not compatible for a medium throughput use, because of their high costs and more complex analysis needs. Gene targeted approaches help reduce costs however they sacrifice the number of genes available for building associations and limit the potential of utilizing Al and deep learning. Here we introduce the high throughput and low cost Clariom™ GO Screen Rat Assay. The assay directly measures 597 rat genes for in vitro and in vivo applications and does not require RNA purification as it can directly utilize cell lysates. The GO Screen uses 384 microarray plate technology, with 3 probes per gene to the most constitutive gene regions. Care was taken to empirically select probes which work on both Sprague-Dawley and Han-Wistar strains of rat. To evaluate the power of the GO-screen assay, liver RNA of rats exposed for 14 days to Phenobarbital (CAR/PXR activator) and a Bayer proprietary compound known to induce liver toxicity, which had previously been analyzed on RAE230 Affymetrix microarrays, was re-run on GoScreen. Comparative analysis revealed that the majority of the overlapping genes showed a similar profile, and their mechanistic interpretation demonstrated that GoScreen delivers the same biology as earlier more expensive microarrays, strengthening our confidence in this novel system. We also explored the use of the Clariom™ GO Screen Rat Assay for early in vitro toxicology screening purposes. In current work, we show the results obtained with primary rat hepatocytes exposed to Clofibrate, a Bayer proprietary compound and betanaphthoflavone, activators of the respective xenobiotic sensing receptors PPARα, CAR/PXR, and AhR. These receptors are known to be Molecular Initiating Events for rodent (non-genotoxic) liver carcinogenesis and liver mediated thyroid toxicity. Unlike AhR, CAR/PXR and PPARα induced rodent liver cancers are thought to be non-relevant for humans, hence the importance for an early tox screen.

Unlike AhR, CAR/PXR and PPARα induced rodent liver cancers are thought to be non-relevant for humans, hence the importance for an early tox screen. GO Screen Rat Assay for early in vitro toxicology screening purposes. In current work, we show the results obtained with primary rat hepatocytes exposed to Clofibrate, a Bayer proprietary compound and betanaphthoflavone, activators of the respective xenobiotic sensing receptors PPARα, CAR/PXR, and AhR. These receptors are known to be Molecular Initiating Events for rodent (non-genotoxic) liver carcinogenesis and liver mediated thyroid toxicity. Unlike AhR, CAR/PXR and PPARα induced rodent liver cancers are thought to be non-relevant for humans, hence the importance for an early tox screen. In summary, the combination of a robust technology, at low cost and covering over 21,000 genes in the rat transcriptome sufficiently covers pathways and GO-terms to realize mechanistic toxicology studies as well as high throughput toxicogenomics as a first entry test for early toxicity assessment in the development of new chemicals.

Mitomycin C (MC) is well known as a DNA alkylation agent via monofunctional and bifunctional alkylation. MC is commonly used to treat cancer with normal p53 activity. The pharmacological mechanism of MC has been analyzed and investigated thoroughly. The MC analog 10-decarbamoyl mitomycin C (DMC), unlike MC, has stronger effects on cancer with the p53 mutation. When the cells are treated with MC, the main DNA inter-strand crosslink (ICL) generated is α-ICL, which possesses a trans stereochemistry. With DMC, a stereoisomeric δ-ICL: an inter-strand crosslink with cis stereochemistry is generated mainly. Unlike MC, DMC’s pharmacological mechanism is not well understood. We previously demonstrated that MC and DMC could activate p27 in MC-7 (p53 proficient) cells and KS62 (p53-deficient) cells. This p27 activation triggered by MC and DMC is p53 independent. p27 and p21 are members of the Cip/Kip family of Cdk inhibitors and crucial as cell cycle regulators. Loss of expression or function of p27 and p21 has been implicated in the genesis or progression of many human malignancies. The purpose of this study is to elucidate the role of p27 in KS62 cells in response to MC and DMC. The results were obtained from the western blot analysis which shows a comparison between the control and varying concentrations of MC and DMC in KS62 cells. A T-Test for two-sample assuming equal variances was performed, and the p values from both one-tail and two-tail were compared. Cells treated with MC at 50 and 75 µM show no significant difference with PBS-treated control cells. However, the cells treated with DMC 50µM were significant with p < 0.05 about 61% reduction. With DMC 75µM, cells also showed much significant reduction in comparison to the control group with p < 0.005 about 61% reduction. There is no difference between the cells treated with 50 µM and 75 µM of DMC. This indicates that DMC triggered stronger effect on p27 expression in KS62 cells after 24 hours exposure. The activation was also found to be p53 independent. Both p27 and p21 are in the same family of CIP/KIP family that inhibits cyclin dependent kinase (CDK) function and regulates the cell cycle. The diminished expression for p27 and p21 has been found to promote the development of many human malignancies. The purpose of this study is to elucidate the role of p27 in MCF-7 cells in response to MC and DMC treatment. The western blot analysis was performed to show the comparison of p27 expression between the control and treated MCF-7 cells. After MCF-7 cells were exposed to 50µM MC for 24 hours the p27 expression on p27 was observed. But a significant change in p27 expression was observed when MCF-7 cells were treated with 50µM DMC with p < 0.05. As for DMC, p27 expression was reduced predominantly in the treated groups of MCF-7 with both 50µM DMC and 75µM DMC. Moreover, the concentration of 75µM DMC had a stronger effect than 50µM DMC. Both MC and DMC can abate p27 expression after 24 hours of exposure in MCF-7 cells. Compared to MC, DMC has a stronger effect on p27 reduction, which was evidenced in the 50µM MC group. The dose-response study of MC and DMC on p27 expression at the lower concentration range should be conducted to reveal a full spectrum of the effects of MC and DMC on p27 expression.
myocytes was evidenced by transmission electron microscopy. Ferroptosis was furthermore confirmed in the heart of mice with DCM. Sulfonaphthrene (SFN) treatment activated NRF2 function, which inhibited AGES-induced ferroptosis in ECTs by upregulating ferritin and SLC7A11 levels. The protective effects of SFN on ferroptosis induced by AGES in ECTs and in the heart of mice with type 2 diabetes were AMPKα2-dependent since the protective effect of SFN on ferroptosis was abolished in AMPK inhibitor-treated ECTs and AMPKα2-KO mice. Hence, ferroptosis plays a role in the pathogenesis of DCM, and SFN prevents ferroptosis and the associated pathogenesis via AMPKα2-mediated NRF2 activation. These findings may suggest a feasible therapeutic approach with SFN for the prevention of DCM in individuals with diabetes.

**2778 Cross Talk between Endonucleases during Acute Toxicity in Kidney Tubular Epithelial Cells**


Enzymatic DNA fragmentation is both an immediate cause and a result of apoptosis and of all other types of irreversible cell death after toxic injury. DNA fragmentation is produced by nine enzymes including DNase I, DNase II, DNase III, their homologs, caspase-activated DNase (CAD) and endonuclease G (EndoG). The endonucleases act simultaneously during cell death; however, regulatory links between these enzymes have not been established. We have previously shown that DNase I and EndoG are the most abundant and the most active apoptotic endonucleases in kidney tubular epithelium. In the current study, we hypothesized that DNase I and/or EndoG may regulate other endonucleases. To test this hypothesis, rat kidney tubular epithelial NRK-52E cells were transfected with the EndoG gene, DNase I gene or DNase I inactive mutant in a pCEP expression vector, while control cells were transfected with the empty vector. mRNA expression of all nine endonucleases was studied using real-time RT-PCR of promoter/exon 1 regions of endonuclease genes. DNA strand breaks in endonuclease genes were determined by PCR; and protein expression of the enzymes was measured by western blotting and quantification by image analysis. Our data show that DNase I is not its inactive mutant, induces all other endonucleases at varying time periods after transfection, causes DNA breaks in promoter/exon 1 regions of endonuclease genes, and elevates protein expression of several endonucleases. The overexpression of mature EndoG in kidney tubular epithelial NRK-52E cells induced expression of CAD and four endonucleases that belong to DNase I group including DNase I, DNase X, DNase II and DNase Y. The induction of DNase I-type endonucleases was also associated with DNA degradation in promoter/exon 1 regions of the endonuclease genes. These results together with findings on colocalization of immunostained endonucleases and TUNEL suggested that DNA fragmentation after EndoG overexpression was caused by DNase I-type endonucleases and CAD in addition to EndoG itself. Overall, these data provide first evidence for existence of the integral network of apoptotic endonucleases regulated by both DNase I and EndoG. We further hypothesize that because the communication between the endonucleases occurs through DNA breaks, the network may be activated by DNA breaks produced by reactive oxygen species, DNA repair endonucleases, or topoisomerases.

**2779 DNA Damage Response Pathways Are Induced after Exposure to Various Heavy Metals in C. elegans**

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A major route of exposure to various heavy metals is through contaminated soil and water. Research has shown that these substances play roles in the induction of various diseases such as cancer, neurodegeneration and birth defects. In the cell, proteins such as metallothioneins respond to heavy metal exposure and chelate the metal to prevent cellular damage. However, little is known about the cellular response in regards to DNA damage after heavy metal exposure. To provide a better understanding of this cellular response, the induction of both cell cycle arrest and apoptosis were investigated after exposure to copper, cadmium, iron, lead, nickel and silver in the nematode C. elegans. Growth assays were conducted to determine EC10 and EC50 concentrations, which were utilized to investigate if apoptosis and/or cell cycle arrest was induced upon exposure. For all metals, apoptosis was induced in the germline with copper, nickel and iron resulting in the greatest induction. To determine if the induction of apoptosis is p53 dependent and thus due to DNA damage, a cep-1 mutant was tested. Apoptosis was induced after exposure to lead and copper at the same level as in the wildtype. Levels were significantly reduced in response to silver, copper, and iron indicating a role for p53 in the induction of apoptosis. Cell cycle arrest was also induced in the

**2780 Impact of Flavored JUUL Electronic Cigarette Liquids on Human Osteoblast-Like Saos-2 Cells**


The use of JUUL, a name brand vaping device, has rapidly become the most popular electronic cigarette (e-cigarette) device among youth since its release in 2015. A study that drive JUUL popularity in the adolescent e-cigarette market, discovered delivery systems, direct marketing tactics, and highly addictive levels of nicotine. There is increasing evidence that points to vaping having adverse effects on human health. This is particularly concerning in adolescents as bone development is still in a critical stage of growth. We are investigating the impact of JUUL electronic cigarette liquids (e-liquids) on bone-forming osteoblasts. We hypothesize that exposure to JUUL e-liquids will impair osteoblast function. Human osteoblast-like Saos-2 cells were exposed to un-vaped JUUL e-liquid concentrations of ranging from 0.01% to 10% for 24 hours and assessed for cytotoxicity using an MTT assay. Compared to a control culture media only control, cytotoxicity was most pronounced in cells treated with 1% menthol flavored JUUL e-liquids (15.6±19.2% viability). Menthol was followed by mango flavor with 76.2±1.9%, Virginia tobacco with 83.8±1.8%, then cherry with 90.1±1.9%. These results indicate a flavor-dependent osteotoxicity with menthol being the most cytotoxic and créer being the least cytotoxic. In ongoing research we are investigating the impact of JUUL exposure on osteoblast extracellular matrix including phosphatase and calcium deposition, as well as type I collagen. Current data includes JUUL e-liquids marketed as 5% nicotine, future experiments will include 3% nicotine JUUL e-liquids. This study provides vital insight in potential ramifications of e-cigarette use by adolescents. This research is supported by an Institutional Development Award (IDeK) from the National Institute of General Medical Sciences of the National Institutes of Health under Grant #P20GM103408.

**2781 Role of Apoptosis and Human Chorionic Gonadotropin Hormone in Bisphenol A-Induced Toxicity in Human Placenta**


Bisphenol A (BPA) is considered a major public health concern. BPA can cross the placenta, potentially affecting human fetal development. Human chorionic gonadotropin (hCG) is a fundamental hormone in the implantation and maintenance of pregnancy. Apoptosis is an essential feature of normal placental development. Placental apoptosis may be initiated by various stimuli, including hypoxia, oxidative stress, and environmental factors. Oxidative stress plays a central role in the pathophysiology of placental function. This study aimed to examine BPA’s effect on the release of β-hCG, the intracellular ROS production, and apoptosis in the human placental trophoblast (BeWo) cells. Cells were treated with BPA at various BPA concentrations or H2O2 as a positive control for different time intervals. The current study assessed Cell viability, cell death, ROS production, level of B-hCG, and apoptosis. BPA significantly reduced cell viability in a concentration-dependent manner after 24h of exposure. Low concentrations (0.0001-1µM) of BPA significantly increased ROS production and apoptosis in a time-dependent manner. At 1µM, BPA-induced apoptosis, indicating that BPA induced apoptosis via the caspase-3 pathway. These results demonstrated that BPA, similar to H2O2, is a potent inducer of apoptosis. The apoptotic effects of BPA, at least in part, are mediated via induction of oxidative stress. BPA displayed a nonmonotonic concentration-response relationship for HCG levels in BeWo cells. BPA at low concentrations of 0.001 to 0.1µM significantly increased HCG levels. However, BPA at higher concentrations (10µM-100,000µM) significantly decreased HCG levels. The decreased β-hCG release indicates loss of trophoblast function. In ECTs by upregulating ferritin and SLC7A11 levels. The protective effects of SFN on ferroptosis induced by AGES in ECTs and in the heart of mice with type 2 diabetes were AMPKα2-dependent since the protective effect of SFN on ferroptosis was abolished in AMPK inhibitor-treated ECTs and AMPKα2-KO mice. Hence, ferroptosis plays a role in the pathogenesis of DCM, and SFN prevents ferroptosis and the associated pathogenesis via AMPKα2-mediated NRF2 activation. These findings may suggest a feasible therapeutic approach with SFN for the prevention of DCM in individuals with diabetes.
Complete and transparent data sharing is a laudable, but challenging goal in toxicity research. Recently, a working group consisting of stakeholders from academia, industry, government, and scientific publishing described a set of guiding principles to make data findable, accessible, interoperable, and reusable (FAIR). These principles are now a cornerstone of data management policies of the NIHES Superfund Research Program (SRP). In order to promote the FAIRness of in vivo toxicity experiments, a working group from several Superfund Research Centers developed the Minimum Information about an Animal Toxicology Experiment (MIATE) requirements and surrounding data management framework which leverages the Investigation, Study, Assay (ISA) data model and related tools. A consensus of essential metadata was established covering animal details (e.g., species), housing conditions, diet, treatment, and study termination conditions. Recommended ontologies for specific fields are used to ensure interoperability. Metadata requirements were defined in relation to the ISA data model to leverage the mature data management ecosystem including the ISA software suite for the collection and validation of metadata. MIATE requirements were also used to develop publicly available data collection templates for ISA tools. We demonstrate the effective implementation of MIATE concomitantly with existing microarray, proteomic, and metabolomic datasets that also adhere to Minimum Information about Sequence Experiments with Genetic Effects (MISEQE). Minimum Information for Proteomic Experiments (MIAPE), and Metabolomics Standard Initiative (MSI) standards. Through use of minimum requirement standards such as MIATE, we hope that data from basic toxicology studies can be standardized to enhance their interoperability and reuse, and consequently improve data capture that is truly FAIR. Funded by Superfund Research Program data interoperability and reuse supplements to MSU (P42ES004911), UK (P42ES007380), UofL (P42ES023716), and UI (P42ES013661).

The Chemical Effects in Biological Systems (CEBS) databases house toxicology study metadata and assay results from National Toxicology Program (NTP) studies. The CEBS-Reporting (CEBSR) data warehouse was designed to integrate data from various legacy databases into one central relational database with the aim to facilitate cross-study data mining. CEBSR contains statistically significant results for histopathology, clinical pathology, reproductive endpoints and other data types. Collating data from legacy databases presented challenges due to the different mechanisms of data collection, and the terms and metadata used to report the data changing over the lifetime of NTP. As part of this effort, these disparate sources were reviewed to harmonize data and create a new data model and related tools. A consensus of essential metadata was established covering animal details (e.g., species), housing conditions, diet, treatment, and study termination conditions. Recommended ontologies for specific fields are used to ensure interoperability. Metadata requirements were defined in relation to the ISA data model to leverage the mature data management ecosystem including the ISA software suite for the collection and validation of metadata. MIATE requirements were also used to develop publicly available data collection templates for ISA tools. We demonstrate the effective implementation of MIATE concomitantly with existing microarray, proteomic, and metabolomic datasets that also adhere to Minimum Information about Sequence Experiments with Genetic Effects (MISEQE). Minimum Information for Proteomic Experiments (MIAPE), and Metabolomics Standard Initiative (MSI) standards. Through use of minimum requirement standards such as MIATE, we hope that data from basic toxicology studies can be standardized to enhance their interoperability and reuse, and consequently improve data capture that is truly FAIR. Funded by Superfund Research Program data interoperability and reuse supplements to MSU (P42ES004911), UK (P42ES007380), UofL (P42ES023716), and UI (P42ES013661).

The chemical characterization of mainstream smoke (MS) from combustible products to understand the potential for harm to human health is challenging because (1) MS may contain hundreds to thousands of distinct chemicals; and (2) it is difficult to predict which chemicals may present significant inhalation toxicity concerns at the observed levels. We have developed a novel procedure to address these challenges in a study of MS from marijuana blunts (MBS). A MBS smoker would be smoked for recreational drug use, is prepared by packing marijuana in a tobacco cigar wrapper, which itself contains tobacco. We performed experiments to compare the chemical constituents of MB MS samples vs. cigar MS samples, where all samples were generated in a laboratory using a linear five-port smoking machine. Our approach provides a possible path forward for developing the larger IERHP comprehensive understanding of individuals' lifetime exposures, and how those exposures may contribute to individual risk. Ultimately, the IERHP model will allow for prioritization of individuals' risk factors, leading to improved hazard protection plans for operational and occupational settings.
2786 Constructing Developmental and Reproductive Toxicity Database of Chemicals, DART NIHS DB, for Integrated Approaches to Testing and Assessment (IATA)


Developmental and reproductive toxicity (DART) is one of the important endpoints in toxicology. Although a number of alternative methods including studies using zebrafish, rat embryos, embryonic stem cells, and pluripotent stem cells are being developed, in silico approaches are expected to be a rapid and cost-effective alternative to assessing the DART of untested chemical substances. To develop a predictive system to identify high-risk industrial chemicals for DART, we first constructed a reliable and transparent DART database (DB), which was named DART NIHS DB, based on the datasets of DART studies of industrial chemicals that were conducted by the Japanese government ministries, Ministry of Health, Labour and Welfare and Ministry of Economy, Trade and Industry. The DB is unique, as all the datasets were created directly from publicly available toxicity study reports conducted under the designated guidelines of OECD TG421 and TG422, and its dataset chemicals have little overlap with those of ToxRefDB which compiles DART data on a large scale. In the DART NIHS DB, 170 of 406 substances showed any signs of DART, and the positive changes occurred from the stages of fertility and early embryonic development (32%), organogenesis (15%), and perinatal (53%). When the lowest observed adverse effect level (LOAEL) of DART was compared with that of repeated-dose toxicity (RDT), 18 substances (11%) showed lower LOAEL of DART than that of RDT. Of these, 5 substances showed significant DART findings at dose levels < 1 ppm, contributing to developing AOP of HDAC inhibition leading to DART endpoint. The whole datasets of the DB can be implemented in the read-across support tool such as OECD QSAR Toolbox, which will further lead to predictive toxicology by read-across and future IATA based on AOP.

2787 Mechanistic Profiling of Compounds with Occupational Exposure Limits Primarily Based on Sensory Irritation


Sensory irritation is often used as a critical effect in setting occupational exposure limits (OELs). For compounds that lack enough toxicity information to set an OEL, occupational exposure banding (OEB) has been used to characterize hazards using incremental bands (< 1 ppm; < 10 ppm; < 100 ppm; < 1000 ppm; ≥ 1000 ppm). There may be different underlying mechanisms across a range of OEBs that can be leveraged to set future values without the need for animal testing. To address this, we applied computational mechanistic profilers to 112 compounds whose OELs were primarily based on sensory irritation (subset of an American Conference of Governmental Industrial Hygienists [ACGIH] database) then categorized the mechanisms across ranking the OEBs by exposure band. Our mechanistic profilers identify neurological compounds, mitochondrial inhibitors, chelants, surfactants and compounds that are chemically reactive. To address sensory irritation, we built a random-forest (RF) machine-learning fingerprint model for the transient receptor potential vanilloid subfamily type 1 protein (TRPV1). TRPV1 was identified as the most likely target across all bands. For 39 compounds banded at < 1 ppm, 33 were flagged for TRPV1 using the fingerprint model but most of these had other structural features, including: being chemically reactive (27), targeting GABA (1), causing methemoglobinemia (1), being acidic/basic (6); or being highly halogenated non-polar compounds (4). Similarly, most compounds flagged for TRPV1 at < 10 ppm were chemically reactive. In contrast at ≥ 100 ppm, compounds potentially targeted TRPV1 but were not chemically reactive or strong acids or bases. Mechanistic profiling confirmed that nociception via TRPV1 is the predominant underlying mechanism associated with those compounds whose OELs were primarily set based on sensory irritation, and demonstrate that the most potent of these compounds (OEB < 10 ppm) can be differentiated based on chemical reactivity and pKa. Compounds flagged for TRPV1 that were chemically reactive may activate a related nociceptor that serves as a sentinel for small electrophiles because of its location at the ligand binding site (transient receptor potential ankyrin 1; TRPA1) [model construct in progress]. Collaborative efforts to build an in vitro screening assay for TRPV1 are welcomed as some of the compounds identified using the RF model were below the molecular weight domain of the control compounds in the training set.

2788 On the Way to Proactive Compound De-risking Using Artificial Intelligence

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Sponsor: R. Bars

Optimization of chemical design aiming at compound de-risking relies on numerous cycles of trials and errors in order to select chemicals with optimal safety profile. In this context, we report generative models that bridge systems biology and molecular design conditioning a generative adversarial network with transcriptomic data or morphological image features. By doing so, we can automatically design molecules that have a high probability to induce a desired biological fingerprint. In a proof of concept, providing biological signature of the desired state, we showed that these models are able to design active-like or non active-like molecules for desired targets without any previous target annotation of the training compounds. Molecules designed by these models are more similar to active compounds than the ones identified by similarity of biological signatures. The same approach in a first instance can be used to design molecules with a reduced probability to interact with specific molecular initiating events causally associated with adverse effects. Overall, this approach represents a new way to bridge chemistry and toxicology. Simulations were done in low toxicity settings, contributing to develop- ing optimized safety profiles. We advocate for a collaborative effort associating industry and academia to join forces in order to build a shared dataset of gene expression and morphological patterns derived from in vitro based test systems.

2789 Determination of Kinetically Derived Maximum Dose (KMD) in Repeated-Dose Studies: A Comparison of Current Statistical Methods

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KMD analysis of toxicokinetic (TK) data from repeated dose toxicity studies is an important step in dose selection for subsequent studies. Existing statistical approaches for KMD aim to define departure from proportionality and at the dose it occurs. To assess performance of each statistical method (quadratic, exponential, ANOVA), 5 simulation models were developed from datasets representative of varying degrees of saturation of elimination, absorption, and toxicokinetics. Simulations were quadratic, power, exponential, log-linearly, log-logistically, and log-hockey-stick models. For each simulation, different settings were created that varied the degree of deviation from dose-proportionality and varied the amount of variance (noise) present in the data. A dose proportional reference line and confidence interval [0.8, 1.25] based on the lowest dose level establishes the Departure Point from Bioequivalent Proportionality (DPBP). Monte Carlo simulations were performed for each combination of model settings, and false positive rate and power were calculated. This excise identified different model scenarios where each method excelled or failed to detect proportionality. The exponential model was found to perform well under most scenarios and tended to be the most sensitive, generally producing a higher rejection rate. It was able to control for false positive rate under the log-linear model for proportionality. The ANOVA approach identified trends that existed within the data, performed well under some scenarios and was able to control for false positives. The quadratic model performed well under clear deviation from proportionality and small variance scenarios but fell short when deviations were not quite as obvious and could not control for false positives. Finally, performance of each statistical method was also assessed using different sample sizes, and different dose windows to simulate capturing different parts of the curve. In conclusion, no one model can be recommended to be used for all KMD studies. Some changes should be considered in the experimental design. Additional work is in progress to improve upon current methodologies to ensure they address both the question of deviation from proportionality, and at what dose level does proportionality deviation become significant.
E-Liquid formulations are typically comprised of nicotine, carrier chemicals (propylene glycol [PG] and glycerol [VGI]), and flavor mixtures. While most flavor ingredients used in e-cigarettes are ‘generally recognized as safe’ (GRAS) for oral consumption, there is limited available information to evaluate their inhalation toxicity. In addition, recent publications that use in vitro assays report some market e-cigarettes (EC) may have adverse toxicity potential. Previously, in vitro to in vivo extrapolation (IVIVE) was performed to translate the in vitro cytotoxicity responses of EC aerosols to human equivalent administered doses (EADs), utilizing reported EC aerosol concentrations of nicotine and flavors (Chang et al, 2020), and found that the human exposures needed to match the in vitro bioactivity exceeds the typical human usage. However, composition data on some major ingredients such as carriers were not available and therefore not included in this analysis. Here we follow up previous IVIVE with the whole product mixture, including carriers and organic acids, to estimate EADs representative of the whole product to support EC risk assessment of e-liquid consumption. Simple steady state and multi-compartment pharmacokinetic (PK) models with different exposure scenarios (2 h and 24 h) were used to evaluate effects of modeling approaches on EAD estimation. MITT cytotoxicity data for e-cigarette aerosol (Omaiyi et al., 2019) were used to predict corresponding human exposure considering a mass balance for the whole product. Using an additive modeling approach, the IVIVE analysis of whole product ingredients in EC aerosols showed up to six-fold higher EAD estimates compared to previous results performed without carriers and organic acids. This is likely because carriers comprise a large volume of the mixture and PG and VG are subjected to extensive intrinsic clearance, reducing their in vivo availability in systemic circulation. While the estimated EADs greatly exceeded typical usage, future studies may evaluate different toxicity endpoints. This case study demonstrates that the pharmacokinetics of whole product ingredients including carriers should be considered when extrapolating in vitro assay data to relevant human exposure to e-cigarettes.

Cardiovascular diseases (CVDs) are a group of disorders of the heart and blood vessels that represent a leading cause of mortality in the United States and worldwide. Various factors influencing CVD have been relatively well-characterized, such as lifestyle choices, genetic factors and off-target pharmaceutical effects. Another potentially significant but underappreciated risk factor contributing to the development and severity of CVD is environmental exposure to chemicals that may interfere with critical CV targets and pathways. The heart and vascular system have been shown to be vulnerable to multiple environmental agents including pesticides, flame retardants, polycyclic aromatic hydrocarbons (PAHs), plasticizers, air pollutants, arsenic, cadmium, lead, and there is mounting evidence that long-term environmental chemical exposure plays a significant role in progression of CVD. To better understand the landscape of environmental chemical influence on CVD, we developed a scoring review to systematically identify and categorize research reporting potential associations between environmental exposures and adverse cardiac outcomes. A comprehensive search was conducted in Pubmed, Scopus, and Web of Science that retrieved over 200,000 references. Given the particularly large number of references, iterative artificial intelligence algorithms were leveraged to prioritize and support manual title and abstract screening in Distiller, and machine learning approaches were used to facilitate categorization of references that reported data on cardiovascular outcomes after exposure to an environmental agent. Relevant references were characterized by evidence strength, human, animal, or in vitro exposure), study design, exposure, and major CV outcomes. An interactive evidence map was prepared to enable researchers to explore data rich and data poor areas in the literature by cardiovascular outcomes, environmental exposures and other key factors. This map will inform evidence-based decisions on the identification, selection, and prioritization of assay platforms and environmental chemicals that will be used by the DNTP to evaluate cardiovascular toxicity.
by EPA’s Office of Pesticide Programs, serve as a primary source for ToxRefDB, along with data from the literature, National Toxicology Program, and pharmaceutical industry. These data and study reports require enormous time, financial, and animal resources but remain largely cached and under-utilized in an image-based format. Our new data collection tool (DCT) is an internal Oracle APEX software developed for curation of additional legacy documents with enhanced quality control and data provenance capabilities. The DCT provides document allocation, curation and workflow management among users, and management review with data conflict resolution, resulting in a record that directly links quality-controlled curation to source documents. The DCT application captures basic study metadata, dose-response, treatment-related and critical effects, and endpoint testing status information while employing controlled vocabulary developed for ToxRefDB. Though the DCT was designed as a ‘one-to-one’ replacement for the previous ToxRefDB document management and curation workflow, with additional customization future versions could support myriad projects that require document management, curational and extracted information. This is an example of one of many tools that will be necessary to promote development of NAMs and data interoperability for computational and regulatory toxicology applications to ultimately achieve reductions in animal testing. This abstract does not necessarily reflect US EPA policy.

2795 Utilizing CDISC SEND Data to Generate Historical Control Incidence from a Large Database of Toxicology Studies

BioCelerate is an industry consortium driving initiatives to increase efficiencies in early stage R&D. The implementation of the SEND model represents an opportunity to apply large-scale data analytics to toxicology data. Before this opportunity can be realized, differences in SEND implementation that make it difficult to conduct cross-study analysis must be addressed. In partnership with FDA, we identified six cross study analysis use-cases and areas in SEND datasets that are significant drivers of variability that negatively impact cross-study analysis. Herein the focus is on the background-control data use case and approaches for improved data harmonization. The most relevant variables to extract from SEND datasets that allow SEND repositories to function as robust and easy-to-use historical control databases have been defined. The US FDA CDER repository of >1,800 SEND datasets was queried to gain insight into how SEND is being applied with respect to these parameters. Proposals for harmonization methods and test search scripts to transform data were developed to allow consistent extraction of these variables across SEND datasets. A framework for the development of proposed solutions from which a user could assemble the results for specific endpoints for all control animals in a user-defined subset of studies is provided. A recent BioCelerate/PHUSE collaboration is engaged in further refinement of SEND database searching and the development of R search scripts for efficient cross-study data analysis. The R search script repository is publicly available on GitHub and can be used to provide greater context into the significance of toxicologic findings observed in toxicology studies. By implementing these proposed approaches, stakeholders will be able to take advantage of the opportunity presented by SEND database to increase efficiency and productivity in early stage R & D, including more robust evaluation of large SEND datasets across multiple studies.

2796 Application of Clinical Benchmarks to NexGen Risk Assessment (NGRA) Decision-Making for Skin Allergy: Use of Historical Clinical Experience to Define Low-Risk Cosmetic Product Market Exposures

We have developed a tiered, exposure-based weight of evidence (WoE) NexGen Risk Assessment (NGRA) framework for Skin Allergy that is based upon International Cooperation on Cosmetics Regulation (ICCR) NGRA principles and aligns with the Cosmetics Europe NGRA framework for Skin Allergy. This framework uses the Skin Allergy Risk Assessment defined approach (SARA DA) to estimate human potency using historical in vivo data [human repeat insult patch test (HRIPT) and mouse local lymph node assay (LLNA, OECD TG 442B) data] and new approach methodology (NAM) data [Direct Peptide Reactivity Assay (DPRA, OECD TG 442c); KeratinoSens™ (OECD TG 442D); human Cell Line Activation Test (hCLAT, OECD TG 442E); U-Sens™ (OECD TG 442E)]. Traditional quantitative risk assessment approaches (QRA) for skin allergy use safety factors to rescale points of departure, such as No Expected Sensitization Levels (NESLs). Using a benchmark approach, we determined safe doses which can be compared against consumer exposure estimates to inform safety decisions. Justifications for the appropriate size of safety factors are drawn retrospectively and largely based upon historical precedent of use. For NGRA, benchmark exposure information may be leveraged to derive empirical support that an exposure is low risk and can be considered safe. To apply this concept to NGRA for skin allergy we established 62 low or high risk benchmark exposures using 10 human contact allergens [methylchloroisothiazoline/methylisothiazolione (MCI/MI), MI, methylidibromogluaronitrile (MDGBN), phenoxyethanol, iodopropynylbutylcarbamate (IPBC), propyl paraben, benzyl alcohol, and sodium benzoate, propyl gallate and hydroxyisooctyl-3-cyclohexane carboxaldehyde (HICC)] with an established history of use in 7 cosmetic products (deodorants, face cream, body lotion, liquid hand soap, shampoo, body wash and lipstick). Benchmark exposure information was then combined with the SARA model predictions to enable a quantitative measure of the risk to the consumer population. Based on these promising initial results, extension of and peer review of the risk benchmarking dataset and approach are now sought.

2797 Developing QSAR Models for Inhibition Growth of Plasmodium falciparum
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Malaria is a severe disease which remains the major cause of mortality in the world. Despite of intensive research and global efforts aimed to prevent, diagnose and treat the disease, 435,000 death cases were reported by the World Health Organization in 2017. The emerging drug resistance to existing drug compounds makes the screen for new drugs the matter of special importance. Malaria may be caused by 6 species of Plasmodium parasite, with Plasmodium falciparum responsible for nearly 90% of deaths occurring in Africa, mostly in children. Our dataset analysis is based on the screen performed in this organism, targeted to kill the parasite by interfering with apicoplast translational machinery. Apicoplast is an essential organelle of Plasmodium falciparum, derived from an engulfed red alga, and considered to be a good drug target due to its evolutionary distance from the host. It was reported that the killing caused by antibiotics targeting the apicoplast is unusual, affecting only second generation of parasites (1), so our assay was based on measuring the growth of parasites, infecting erythrocytes, incubated with tested compounds for 48 and 96 hours. Compounds targeting the apicoplast should inhibit parasite growth in prolonged incubation only, representing the second generation of the parasite. The measurements of Plasmodium growth were performed by detection of luciferase reporter integrated into the parasite cells. The dataset with 996 compounds was acquired from PubChem (aid 504848) and literature and was carefully curated based on OECD principles. Different algorithms were used for models: Regression, Random Forest, Naïve Bayes, k-Nearest Neighbors. Bootstrapping. Leave 10% out, and Y Scrambling were used for model validation. The best model shows an excellent predictive performance of 92% ± 6 sensitivity, 92% ± 7 specificity, 92% ± 9 positive accuracy and 90 % ± 8 negative accuracy by the leave-group-out method and 94% ± 7 sensitivity, 94% ± 7 specificity, 93% ± 9 positive accuracy, and 94% ± 6 negative accuracy by the bootstrap. The model has identified 6 structure alerts.

2798 Estimating EC3 (Effective Concentration for a Stimulation Index of Three) Confidence Bounds and Uncertainties for Skin Sensitization Based on Structure Similarity and Assay Profiles
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When reliable toxicity data for a target substance are not available to address its skin sensitization potential, non-animal testing alternative methods now engage in silico methods such as structural alerts, QSARs and in chemico / in vitro assays based on the published Adverse Outcome Pathway (AOP). Read-across based on structural or mechanistic analogs is also receiving at-
2799 Application of Open-Source PBPK Models in Rat-to-Human Pharmacokinetic Extrapolation of Oral Nicotine Exposures

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Physiologically based pharmacokinetic (PBPK) models allow chemical-specific modeling of chemical movement (Administration-Distribution-Metabolism- Elimination; ADME) through the body. PBPK models are often developed based on animal data and applied to predict ADME in humans, considering interspecies differences in physiology or exposure scenarios. In this study, we first utilized an open-source PBPK model to simulate rat plasma nicotine concentrations following 7-day gavage dosing of nicotine formulations. We modeled up to 8 mg nicotine/kg/day, which elicited no acute toxicities based on in-life and histopathological evaluations. We then used the optimized rat PBPK model to predict human-relevant nicotine plasma concentrations from oral nicotine product (gum) exposures. When extrapolated to human oral nicotine exposures, the rat-optimized gavage model underpredicted the maximal plasma concentration (Cmax) of nicotine as measured during human use. Lacking experimental data from rat “buccal” dosing, we simulated human kinetic profiles after nicotine gum exposure using the rat model with gut absorption adjustment (“oral-absorption adjusted”). A 6.4-fold increase in the gut absorption rate in the rat model is needed to fit human plasma data from nicotine gum use. That is, the simulated results showed a nicotine Cmax ~3-5-fold higher in the human nicotine gum users than in the gavaged rats for the same daily dose. We hypothesized that this apparent discrepancy in Cmax and the absorption rate is due to the difference in the route of nicotine uptake between rat and human (nicotine subjected to first pass metabolism prior to entering plasma) and human oral product uses (nicotine absorbed directly via buccal uptake). To approximate the impact of bypassing initial liver metabolism, we used the “IV-buccal adjusted” model to correct for partitioning of nicotine directly from the mouth to the plasma. Literature information on nicotine extraction and bioavailability from oral nicotine products was used to inform the buccal adjustment parameters. The “IV-buccal adjusted” model results were comparable to the “oral-absorption adjusted” simulation and approximated the human data. These results suggest understanding species-specific nicotine product characteristics, dosing routes and uptake mechanisms are critical in estimating human relevant exposure from animal experiments.

2800 Update on the ToxCast Pipeline and Invitrodb


The US Environmental Protection Agency ToxCast data pipeline (tcpl) for high-throughput toxicity testing (HTT) has been applied to nearly 1900 hundred assay endpoints covering diverse biological targets from distinct proteins to more complex cellular processes like mitochondrial toxicity, nuclear receptor signaling, immune response, and developmental toxicity. As open source R package software, tcpl enables first tier data processing of heterogenous assay activity data from HTT, including curve-fitting and estimation of potency and efficacy values. The primary objective of this presentation is to describe major updates to tcpl and the results database, invitrodb, to increase assay annotation, update functions for cytotoxicity threshold estimation, expand curve-fitting models, and enhance plotting capabilities. The number of publicly available assay endpoints in the upcoming version 4 release has increased to nearly 600 assays, 1300 components, and 1900 endpoints, with updates in assay annotations to enable better data aggregation. The lower bound estimate of the cytotoxicity threshold has been updated, requiring a 5% positive rate in curated cytotoxicity assays (now totaling 88). Tcpl curve-fitting models (constant, Hill and gain-loss models) have been expanded to include Polynomial 1 (Linear), Polynomial 2 (Quadratic), Power, Exponential 2, Exponential 3, Exponential 4, and Exponential 5 based on BMDExpress and encoded by R package dependency tcplFit2. Inclusion of these models impacted invitrodb in two primary ways: (1) the need for long-format storage of genetic modeling and the need to focus on selection of winning model. Continuous hit call probability in tcplFit2 is calculated as the product of three different probabilities: median response and top of model exceed the cutoff, and AIC is less than the constant model. Plotting in tcpl has been expanded with a new utility called tcplPlot that provides interactive display of concentration-response curves through plotly integration with built-in REST api functionality. Integrating tcplPlot with tcpl allows for a consistent visualization of curves in reports and web applications. Tcpl and invitrodb provide a standard for consistent and reproducible curve-fitting and data management for diverse in vitro assay data with readily available documentation, thus enabling the sharing and use of these data in myriad toxicology applications. This abstract does not necessarily reflect US EPA policy.

2801 Developing a Feature Selection Pipeline for Target-Based Prediction of Drug Toxicity


A central goal of computational toxicology is to predict in vivo toxicity of a chemical from its structure. Existing methods such as Quantitative Structure-Activity Relationship (QSAR) models are limited by low accuracy and interpretability. To address the issue, we introduce a pipeline that can identify target proteins predictive of drug toxicity. Our pipeline relates structure properties to toxicity outcome via the target binding profile of drugs. We incorporated a feature selection method named ReBATE in the pipeline, which enables the identification of predictive targets. Previous benchmark studies showed that ReBATE outperforms other feature selection methods in detecting genotype-phenotype associations. We implemented our pipeline to train QSAR models on 569 datasets from diverse sources as drug toxicity data of 139 adverse events from OFFSIDES. We demonstrated that our pipeline can accurately predict the binding outcomes of 364 targets (Area Under the Receiver Operating Characteristic curve, AUROC > 0.85), as well as the toxicity outcomes of 18 adverse events (AUROC > 0.65). We also found that feature selection significantly improves the performance of our pipeline (P-value = 0.01). For each adverse event, we identified 30-70 targets that are predictive of the outcome. We found these identified targets tend to be differentially expressed in the matched tissue, according to GTEx expression data. This finding suggests the identified targets participate in tissue-specific functions. We also found direct evidence that connected identified targets to disease markers, and therapeutics. Our findings will gain insight into the cellular mechanisms underlying structure-toxicity associations.

2802 OrbiTox: A Translational Discovery Platform for Concerted View and Analysis of Big Data

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OrbiTox is an interactive 3D visualization and analysis platform for big data from varied scientific domains with special emphasis on predictive toxicology. An intuitive and immersive in-silico environment, paired with tens of millions of experimental and modelled data-points, makes OrbiTox one-of-a-kind enabling knowledge extraction through chemical, genetic, pathway, and in-vivo data. It also offers data gap-filling predictive models and specialized cheminformatics tools for in-silico profiling of chemicals and read-out assessments. To enable translational discovery via a collective view of data from different domains and their interrelationships, OrbiTox projects high-dimensional data from different domains (chemical, gene, pathway, species) in concentric 3D globes. The Chemical globe contains ~800,000 substances (DSSTOX) with structure, carcinogenicity, mutagenicity, and Tox21 qHTS data from public sources (CPDB, IARC, NTP, ToxNet). Similarly, ~25,000 fully annotated human genes and ~2000 pathways with functional annotations are populated in the Gene and Pathway globes respectively. The Species globe contains data from varied animal studies. To enable predictive toxicology applications, we have also included QSAR models built using SAAGAR descriptors which are interpretable 834 sub-structures. We show how OrbiTox can help readily visualize, for example, which pathway is related to which...
driven by a complex interplay of DNA flanking sequence and local chromatin highly predictive of AhR binding within and across MCF-7 and HepG2 cell Our results demonstrate highly accurate and robust models of within-tissue treatment), and non-induced AhR in HepG2 hepatocellular carcinoma cells. DRE proximity to gene promoters. In this study, we predicted AhR binding of and transcription factor (TF) ChIP-seq signals, as well as 3) features denoting the DRE, and 2) local chromatin context, such as DNase-seq, histone mark of combining features representing 1) DNA sequence immediately flanking architecture, to predict bound DREs by investigating non-linear effects expression. Our goal was to develop interpretable predictive machine learning models of DRE-centered AhR binding with the aim of accurate cross-tissue prediction. To this effect, we applied XGBoost, a supervised machine learning architecture, to predict bound DREs by investigating non-linear effects of combining features representing 1) DNA sequence immediately flanking the DRE, and 2) local chromatin context, such as DNase-seq, histone mark and transcription factor (TF) ChIP-seq signals, as well as 3) features denoting DRE proximity to gene promoters. In this study, we predicted AhR binding of exogenously induced AhR in MCF-7 human breast cancer cells (45 minutes or 24 hours of TCDD treatment), human primary hepatocytes (24 hours of TCDD treatment), and non-induced AhR in HepG2 hepatocellular carcinoma cells. Our results demonstrate highly accurate and robust models of within-tissue binding, verified for generalization through 5-fold cross validation. Our results further indicate several specific transcription factors and histone marks as highly predictive of AhR binding within and across MCF-7 and HepG2 cell lines. Additionally, we show that tissue-specific AhR binding appears to be driven by a complex interplay of DNA flanking sequence and local chromatin context features, as well as other tissue-specific mechanisms.

**2803 Accurate Tissue-Specific In Silico Genome-Wide Prediction of Aryl Hydrocarbon Receptor Binding**
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The aryl hydrocarbon receptor (AhR) is an inducible transcription factor with various exogenous ligands, including the potent environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and other similar chemicals (dioxins). Dioxin-mediated toxicity is achieved through AhR activation and binding to the DNA, chiefly the canonical 5'-GGGTG-3' binding motif known as Dioxin Response Element (DRE). Importantly, in vivo AhR binding in human tissues is highly dynamic and tissue-specific. Approximately 50 percent of the experimentally verified binding sites do not contain a DRE, and a great number of otherwise accessible DREs are not bound by AhR. Identification of the drivers and determinants of AhR binding, especially underlying tissue specificity, is crucial for our understanding of downstream gene regulatory effects and potential. In these health outcomes such as liver toxicity and immune suppression. Our results demonstrate highly accurate and robust models of within-tissue binding, verified for generalization through 5-fold cross validation. Our results further indicate several specific transcription factors and histone marks as highly predictive of AhR binding within and across MCF-7 and HepG2 cell lines. Additionally, we show that tissue-specific AhR binding appears to be driven by a complex interplay of DNA flanking sequence and local chromatin context features, as well as other tissue-specific mechanisms.

**2804 The Genotoxicity of Pyrrolizidine Alkaloids Is Mediated by CYP3A in Metabolically Competent TK6 Cell Lines**
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Pyrrolizidine alkaloids (PAs) are widely found in plants. Humans can be exposed to PAs through the consumption of PAs-containing food, plants, or herbal products. PAs have been shown to be genotoxic after metabolic activation. In the present study, we adopted a battery of fourteen recently established human lymphoblastoid TK6 cell lines, each expressing a single human cytochrome P450 (CYP1A1, 1A2, 1B1, 2A6, 2B6, 2C8, 2C18, 2C19, 2D6, 2E1, 3A4, 3A5, and 3A7), and identified specific CYPs responsible for the bioactivation of three PAs - lasiocarpine, riddelliine, and senkirkine. When compared to the empty vector control cell line, all three PAs had increased cytotoxicity in TK6 cells expressing CYP3A4. LC-MS/MS analysis revealed the formation of 1-hydroxymethyl-7-hydroxy-6,7-dihydroprroline (DHP), the main reactive metabolite of PAs, in CYP3A4-expressing TK6 cells. To a much lesser extent, DHP was also detected in CYP3A5- and 3A7-expressing cells after PA exposure. To examine the genotoxic potential of PAs after metabolic activation, we used a high-throughput micronucleus assay and demonstrated that they induced concentration-dependent increases in micronuclei and G2/M phase cell cycle arrest in three CYP3A4-varying TK6 cell lines. We further observed that PA-induced apoptosis, cell cycle changes, and DNA damage were predominantly mediated by CYP3A4 by measuring the protein levels of cleaved caspase 3, pCHK1, pCHK2, and yH2AX. Finally, Bayesian benchmark dose (BBMD) modeling showed that lasiocarpine was the most potent inducer of micronuclei among the three investigated, with a BMD100 of 0.036 μM. In sum, these results indicate that the newly developed TK6 cell system holds promise for genotoxicity screening of compounds requiring metabolic activation, identifying specific CYPs involved in bioactivation, and discriminating genotoxicity potency of PAs that have different structural PA types.

**2805 Synergistic Genotoxicity of Alcohol and Benzo[a]pyrene in HepG2 Cells**

Alcohol use accounts for about 6% of all cancers and 4% of all cancer deaths in the United States according to American Cancer Society; it has been linked with several cancers, including those of the mouth, liver, and breast. The underling mechanisms for alcohol carcinogenesis are still unclear. Multiple factors can be at play: alcohol is metabolized into acetaldehyde, a genotoxic chemical that can cause cancer in laboratory animals; and/or that alcohol increases reactive oxygen species (ROS), leading to DNA damage and lipid peroxidation, increasing the risk of cancer. However, alcohol has not been proven genotoxic. Although synergistic effects of combining drugs and alcohol have been widely described, their combination relative to genotoxicity endpoints has not been previously reported. In this study, HepG2 cells were treated with different concentrations of alcohol and benzo[a]pyrene (BaP) separately or in combination. The genotoxicity was evaluated using the Micronucleus Assay and the measurement of ROS to explore possible synergistic genotoxicity when combining alcohol and genetic agents. Co-treatment of 50Mm alcohol with 1 or 5μM BaP induced significantly higher ROS than either chemical alone. Micronucleus Assay results showed a higher genotoxic sensitivity of the combination, 25%M alcohol with 1μM BaP inducing significantly higher micronuclei than 1μM BaP alone. Increased concentration of BaP and alcohol resulted in increases in MN in a dose-dependent manner. As in previous reports, the combination of alcohol and BaP caused a significant increase in lipid staining in HepG2 cells compared to alcohol alone, demonstrating a synergistic ROS effect. Currently, enzyme modified Comet Assays are being performed to demonstrate specifically the possible effect on the combination of alcohol and BaP and has on the overall higher sensations of BaP and alcohol, possibly via increasing total oxidative stress in HepG2 cells.

**2806 Performance of Metabolically Competent HepaRG Cells for Genotoxicity Testing: A Comparison between Three Human Hepatic Cells Using the High-Throughput Micronucleus and Comet Assays**

Genotoxicity testing plays an essential role in hazard identification and carcinogenic risk assessment. The traditional genotoxicity assays are usually conducted in cells lacking metabolic competency or using rat s9 as a metabolic system, which is an important drawback of the current in vitro systems for genotoxicity assessment. The present study evaluated the performance of HepG2, metabolically competent HepaRG cells, and primary human hepatocytes (PHHs) in the high-throughput in vitro micronucleus (MN) and/or comet assays for genotoxicity testing. A set of 28 compounds with known genotoxic or carcinogenic modes of action (MoA) were tested. The results showed that HepG2 cells had low or undetectable CYP450 enzyme activities compared to PHHs and HepaRG cells, and the CYP activity levels were comparable between HepaRG and PHHS. In the MN assay, both HepaRG and HepG2 cells were treated with 12 genotoxic (seven direct and five indirect) carcinogens for 24 h and cultured for additional hours to go through 1.5-2 cell cycles. HepaRG cells exhibited a higher sensitivity (83%) than HepG2 cells (67%) in detecting the 12 indirect-acting genotoxicants or carcinogens. In the comet assay, following a 24-h treatment, PHHH and PHHS had no effect on overall higher sensitivity (90%) for detecting DNA damage from 11 genotoxicants or carcinogens than HepaRG (70%) and HepG2 (60%) cells. HepaRG cells showed a 100% specificity in detecting 16 non-carcinogens in both the MN and comet assays. The combination of both assays showed 93% accuracy and 100% specificity in detecting genotoxic potential of the 28 compounds. In addition, HepaRG cells correctly predicted the genotoxic MoA of 8 compounds using the Multiflow DNA Damage Assay. These results suggest that HepaRG cells can be adapted for high-throughput flow cytometry-based MN and the CometChip assays for genotoxicity assessment, and that HepaRG cells appear to be more sensitive than HepG2 cells in detecting genotoxicants or carcinogens that require metabolic activation.
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2807 Development of a Three-Dimensional (3D) In Vitro Model from Metabolically Competent HepaRG Cells to Evaluate Chemical-Induced Genotoxicity Using the High-Throughput CometChip Platform

J. Seo, X. He, and X. Guo, US FDA/NCTR, Jefferson, AR.

Human liver cells have been proposed as an in vitro model for genotoxicity assessment as many human carcinogens require metabolic activation to elicit the genotoxicity. Our previous study demonstrated that metabolically competent HepaRG cells detected a large portion of genotoxicants or carcinogens that require metabolic activation. In recent years, three-dimensional (3D) cell culture systems have been increasingly recognized as a better model than traditional 2D monolayer cells for mimicking human in vivo exposures due to improved cell-to-cell interactions and structures. However, applying 3D cell culture in high-throughput genotoxicity assays remains challenging. In the present study, we developed and optimized a 3D culture system by plating fully differentiated HepaRG cells in ultra-low attachment plates (96- or 384-wells). The characterization study showed that 3D HepaRG spheroids expressed higher levels of albumin and CYP450 enzyme activities than their 2D counterparts over a long period of 30 days. Three-D spheroids at day 10 after seeding (300-500 µm in diameter) were exposed to various concentrations of 35 test articles, including direct-acting and indirect-acting carcinogens as well as compounds that show different genotoxic responses in vitro and in vivo. Following a 24-h treatment, the cytotoxicity and DNA damage potential was evaluated by the ATP assay and the high-throughput CometChip assay, respectively. Differences in DNA damage response (positive or negative) were observed between 2D and 3D cultures. Overall, improved sensitivities for detecting DNA damage response were observed in 3D spheroids than in 2D cultures. The results demonstrated that 3D HepaRG spheroids can be successfully adapted to the high-throughput CometChip assay for genotoxicity testing. We anticipate that the application of 3D HepaRG spheroids in high-throughput genotoxicity assays can provide an efficient way for better predicting human in vivo responses.

2808 TubulinTracker, a Novel In Vitro Reporter Assay to Study Intracellular Microtubule Dynamics, Cell Cycle Progression, and Aneugenicity


Aneuploidy, the presence of an abnormal number of chromosomes, can be caused by any process that interferes with chromosome segregation during mitosis, including microtubule disruption or inhibition of cell cycle kinases, like Aurora A/B/C. To detect aneugenicity, as result of chemical exposure, typically the micronucleus assay is used. However, both broken DNA fragments caused by clastogenic agents and mis-segregated chromosomes caused by aneugenic agents can lead to the micronucleus formation. To confirm an aneugenic mode of action, a laborious centromere or FISH staining is required. To improve the detection of genotoxic compounds with an aneugenic mode of action, we developed a novel in vitro assay using GFP-tagged tubulin, that allows the direct visualisation of microtubuli to follow the dynamics during the cell cycle using either live cell microscopy or the quantification of stably bound GFP-Tubulin using flow cytometry. First a GFP-Tubulin reporter was stably integrated into mouse embryonic stem cells. We confirmed that GFP-Tubulin expression did not adversely affected microtubule function, cell cycle progression or genome stability. Next, the GFP-Tubulin reporter cells were exposed to various Tubulin poisons and the GFP-tubulin signal in polymerised microtubuli was quantified using flow cytometry. A DNA staining was included to assess the effect of the agents on the cell cycle progression induction. The GFP-Tubulin reporter assay could discriminate between tubulin stabilising and Tubulin destabilising substances as well as detect mitotic defects. Treatment with agents that affect cell cycle progression but not microtubule stability, such as DNA damaging agents or Aurora kinase inhibitors, did not affect tubulin stability. Additionally, the reporter cell line was used to assess the effect of microtubule poisons over time with microscopy, using the same extraction method to visualise the microtubules. In conclusion, the novel microtubule dynamics reporter assay, TubulinTracker, can efficiently detect both stabilising and destabilising microtubule disruptors and can be used to detect an aneugenic mode-of-action of genotoxic compounds. Insight into the MOA provided is important for hazard identification and as part of AOP and weight of evidence approaches.

2809 A GLP Validation of the 3D Skin Comet Assay as an Alternative to Animal Testing


Skin is the first route of exposure to a variety of compounds found in cosmetics, households products and industrial chemicals. Although the revised OECD Testing Guideline has highlighted the importance of considering in vitro skin models when investigating genotoxic hazard, until now, in vivo assays are still the most common approach to address dermal genotoxicity. The 3D Skin Comet Assay is a good alternative to bridge the gap between in vivo and in vitro testing in terms of final hazard assessment, as it also addresses two underrepresented aspects in classical in vitro genotoxicity assays, i.e. species-and organ-specific xenobiotic metabolism and the relevant route of exposure. To date, the European Regulators have accepted data from the 3D Skin Comet Assay as ‘weight of evidence’ to consider as ‘safe to use’ based on dossiers submitted for three different hair dye ingredients. The objective of this study was to perform a GLP validation of the 3D Skin Comet Assay, using Phenion Full-Thickness Skin Models. The skin models were either untreated, topically exposed to acetone (negative control), a direct mutagen methanesulfonate (MMS at 5 µg/cm²), or treated with an indirect mutagen benzo[a]pyrene (BaP at 12.5 µg/cm²) in the presence or absence of Aplidin [ApliCol] an inhibitor of DNA polymerases that induces accumulation of DNA strand breaks and amplifies comet formation. The topical exposure was performed at 3, 24 and 48 h prior to processing for analysis. Three independent experiments were performed using triplicate skins. Cytotoxicity was measured by distinct endpoints: (i) ApliCol-induced DNA damage; (ii) the intracellular ATP assay; cell energy status. Both assays confirmed high viability in all tested conditions. To address the extent of skin DNA damage, the epidermis was separated from dermis to prepare single cells suspensions, and the % Tail DNA was evaluated in each separate compartment in the comet assay. The results demonstrated in all the 3 occasions, low levels of DNA damage (% Tail DNA (range): 3.20-10.19 in the non-treated (epidermis: 7.36-14.71) and acetone-treated (epidermis: 8.16 -14.09, dermis: 3.98-11.25) samples, while a statistically significant increase was obtained after treatment with MMS (epidermis: 35.96-38.14, dermis: 28.60-40.03) and BaP (in the presence of ApliCol) (epidermis: 30.01-40.03 (3D-LOC-PHH) and 30.01-40.03 (3D-LOC-HepaRG)) when compared to the negative control. The validation has shown that the 3D skin comet assay is a reproducible, accurate and robust method that could be an useful tool to address in vitro skin genotoxicity as an alternative to animal testing.

2810 Application of a Liver-on-Chip Integrated with Human Lymphoblastoid Cells (TK6) to Genotoxicity Testing: Go with the Flow and Reduce Animal Testing


The current in vitro pre-clinical genetic toxicology test battery includes the use of a metabolic activation system (induced rat S9 mix) single cells and bacteria to detect drug mutagenicity and drug-induced DNA damage, respectively. However, a considerable amount of drug development failure is due to unexpected toxic effects that are often related to liver biotransformation. The application of Liver-on-Chip (LOC) technology to genotoxicity testing could potentially result in a more predictive human outcome. The objective of this study was to validate a three-dimensional (3D) organ microchip concept of a human competent metabolic LOC that may be co-culture with human lymphoblastoid TK6 cells, and therefore be applied to genotoxicity assessment. The LOC are comprised with primary human hepatocytes (PHH-LOC) or HepaRG cells (HepaRG-LOC) and TK6 cells. The system allows for communication between two compartments and the performance of multiple endpoints analysis, i.e. the comet assay (% tail DNA intensity) in liver cells and the micronucleus (MN) assay in TK6 cells. Both LOC (extracellular matrix, endothelial cells, PHH or HepaRG and matrigel) were maintained under CTN BIO® microfluids flow technology, with TK6 cells cultured on top in transwells. Both compartments were treated for 0, 24 and 45 h with either negative control, a direct mutagen methanesulfonate (MMS at 5µg/mL) or with indirect mutagens benzo[a]pyrene (BaP at 10µg/mL) and cyclophosphamide (CP at 50µg/mL). Three independent experiments were performed and the liver and TK6 cells were collected. The results demonstrated low levels of DNA damage (% tail DNA intensity) in the negative control (0.10 (3D-LOC-PHH) and 0.07 (3D-LOC-HepaRG)) and a significant increase with MMS (8.82 (3D-LOC-PHH) and 7.39 (3D-LOC-HepaRG)) and BaP (15.02 (3D-LOC-PHH) and 12.32 (3D-LOC-HepaRG)). No increase of % tail DNA intensity was observed after treatment with CP. However, increase in % MN after treatment with CP (76.80 (3D-LOC-PHH) and 3.3 (3D-LOC-HepaRG)), MMS (124.73 (3D-LOC-PHH) and 124.73 (3D-LOC-HepaRG)) and BaP (124.73 (3D-LOC-PHH) and 124.73 (3D-LOC-HepaRG)) and BaP (124.73 (3D-LOC-PHH) and 124.73 (3D-LOC-HepaRG)) and BaP
2811 Identifying DNA Repair Genes That Confer Resistance to P450-Activated Carcinogens Using a Humanized Yeast Library

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Saccharomyces cerevisiae (budding yeast) deletion libraries can be used to categorize specific drug targets, and identify genes that confer resistance to DNA damaging xenobiotics, such as aflatoxin B1 (AFB1). While the original library consists of more than 5,000 strains, each containing a unique gene deletion, we are currently using 175 genes that have been classified as DNA repair genes. To identify genes that confer drug resistance, the pooled library is exposed to the xenobiotic and molecular barcodes and are sequenced using the Illumina HiSeq platform. Since many yeast genes are orthologous to human genes, the overall goal is to identify xenobiocic resistance genes in yeast, and determine whether the corresponding human gene also confers resistance to the particular xenobiotic. Many chemicals and pharmaceuticals, however, require activation by human cytochrome P450 (CYP) enzymes, and we are now introducing CYP3A4, which metabolizes 50% of all pharmaceuticals, into our collection. Using current transformation protocols, the process of introducing CYP genes into yeast libraries is time-intensive and inefficient. Through the use of selective ploidy ablation (SPA), we successfully demonstrated that CYP3A4 expression vector can be transferred into a subset yeast deletion strains. SPA involves a donor strain containing the expression vector in which the centromeres can be destabilized by galactose inducible RNA transcripts and are marked with a URA3 gene. After mating with the donor strain, the resultant diploid is inoculated in galactose media and then in 5-fluorouracil acid (FOA) medium which selects against the donor strains chromosomes. DNA repair genes, such as mismatch repair genes and those that stabilize DNA replication forks, have been identified as AF131-gene resistance. These libraries thus provide a means to rapidly identify yeast DNA repair genes that confer resistance to CYP-activated xenobiotics.

2812 Analysis of Genetic Susceptibility Factors for N-nitrosamine-Induced Toxicity, Genomic Instability, and Cancer

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N-nitrosodimethylamine (NDMA) is a genotoxic IARC Group 2A probable human carcinogen. NDMA contamination from the Olin Chemical Superfund site led to widespread exposure in Wilmington, MA, and recently several pharmaceuticals have been found to contain high levels of NDMA. One major DNA lesion caused by NDMA is the replication-blocking 3-methyladenine (3MeA), which is excised by the alkyladenine DNA glycosylase (AAG) to initiate Base Excision Repair (BER). Notably, since 3MeA blocks replicative polymerases which is excised by the alkyladenine DNA glycosylase (AAG) to initiate Base Excision Repair (BER). Notably, since 3MeA blocks replicative polymerases, the particular xenobiotic. Many chemicals and pharmaceuticals, however, require activation by human cytochrome P450 (CYP) enzymes, and we are now introducing CYP3A4, which metabolizes 50% of all pharmaceuticals, into our collection. Using current transformation protocols, the process of introducing CYP genes into yeast libraries is time-intensive and inefficient. Through the use of selective ploidy ablation (SPA), we successfully demonstrated that CYP3A4 expression vector can be transferred into a subset yeast deletion strains. SPA involves a donor strain containing the expression vector in which the centromeres can be destabilized by galactose inducible RNA transcripts and are marked with a URA3 gene. After mating with the donor strain, the resultant diploid is inoculated in galactose media and then in 5-fluorouracil acid (FOA) medium which selects against the donor strains chromosomes. DNA repair genes, such as mismatch repair genes and those that stabilize DNA replication forks, have been identified as AF131-gene resistance. These libraries thus provide a means to rapidly identify yeast DNA repair genes that confer resistance to CYP-activated xenobiotics.

2813 In Vitro Mutagenicity Evaluation of Commercial JUUL Product E-liquids and Aerosol Condensates

Yao. JUUL Labs Inc., San Francisco, CA. Sponsor: A. Ayala-Smith

To help understand the health risks associated with the JUUL Electronic Nicotine Delivery System (ENDS) products, four JUUL ENDS products and the reference 3RF cigarette were tested for mutagenicity in the in vitro Ames assay, with and without metabolic activation (S9) according to OECD TG471. All four JUUL ENDS products was tested as an e-liquid and as aerosol condensates. The condensates were collected on a Cambridge filter pad followed by an ethanol containing impinger in an ice bath, using intense and non-intense puffing regimes. Cigarette smoke was generated using the ISO 20778:2018 puffing regimen and the smoke condensate was collected in a similar apparatus as for the ENDS condensate. In the Ames assay, the 3RF smoke condensate tested positive in strains TA98, TA100 and TA1537 at a concentration of 102.5 and 2ulate, respectively. In contrast, the e-liquid and the condensates from all JUUL ENDS were negative in all strain tested, up to the highest nicotine concentrations; 340 µg /plate. In summary, the four JUUL ENDS e-liquids and aerosol condensates are not mutagenic under the tested conditions.

2814 Adopting Duplex Sequencing Technology for Genetic Toxicity Testing: A Proof of Concept Mutagenesis Experiment with N-Ethyl-N-Nitrosourea (ENU)-Exposed Rats

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Duplex Sequencing (DuplexSeq) is an error-corrected sequencing method in which molecular barcodes are used to preserve the origin of PCR-copied DNA strands, enabling precise identification and removal of errors arising during amplification and sequencing by comparing grouped strand sequences. The resulting background of one artificial mutation per 108 nucleotides allows detection of true, somatic mutations. TwinStrand Biosciences is developing a Duplex Sequencing Rat Mutagenesis Assay to sample the entire rat genome in an unbiased, representative manner, for use in genotoxicity testing. For the assay, a 48 kb panel is used to target 20 arbitrary regions across 18 of 20 rat autosomes; each hybrid selection bait is about 2.4 kb. To evaluate the sensitivity of the assay for early detection of mutagenesis, male Hsd:Sprague Dawley SD rats (3/group) received a single oral gavage administration of 40 mg/kg N-ethyl-N-nitrosourea (ENU), and mutation frequency and spectrum were analyzed in blood, bone marrow, liver, and stomach tissues at 3 h, 24 h, 7 days, and 28 days post-exposure. In vehicle control rats (sampled at 28 days), mutation frequency and spectrum were similar for all four tissues. Significant increases in mutation frequency were observed in ENU-exposed rats as early as 3 and 24 h for stomach and bone marrow, respectively, and at 7 days for liver and blood. The mutational signature of ENU - increases in T-A and T-C mutations - was established by 7 days post-exposure in all four tissues. However, an increased fraction of non-canonical C-T mutations was observed in all four tissues at the two earlier timepoints. These results demonstrate that DuplexSeq can detect significant increases in chemical-induced mutations within 24 h of exposure and can monitor the evolution of mutational spectra over time. Additionally, an interlaboratory reproducibility study using a subset of the samples showed a Pearson perfect correlation (R = 0.986) for mutation frequency, and consistency in mutation spectra, between TwinStrand and ILS, LLC, demonstrating the transferability of the technology to research laboratories.

2815 Biomarkers of DNA Damage Response Improve In Vitro Micronucleus Testing by Providing Genotoxic Mode of Action and Reducing Occurrences of False Positive Results

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We have previously described two flow cytometry-based in vitro genotoxicity tests: micronucleus scoring (MicroFlow), and a multiplexed DNA damage response biomarker assay (MultiFlow). Here, we describe an approach for integrating the assays in order to efficiently supplement micronucleus analyses

(6.33 (3D-LOC-PHH) and 3.6 (3D-LOC-HepaRG)) were observed when compared to the negative control (2.6 (3D-LOC-PHH)) and 1.72 (3D-LOC-HepaRG)). In conclusion, both LOC showed metabolizing properties and the capability of addressing two genotoxic adverse outcomes within one system, i.e. induction of chromosomal damage or damage to the mitotic apparatus (micronucleus test) and DNA strand breakage (comet assay).

2816 Analysis of Genetic Susceptibility Factors for N-nitrosamine-Induced Toxicity, Genomic Instability, and Cancer

Results

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We have previously described two flow cytometry-based in vitro genotoxicity tests: micronucleus scoring (MicroFlow), and a multiplexed DNA damage response biomarker assay (MultiFlow). Here, we describe an approach for integrating the assays in order to efficiently supplement micronucleus analyses
with a constellation of biomarkers that comment on cytotoxicity (i.e., gamma-H2AX, phospho-histone H3, p53, and cleaved parp) and a constellation of biomarkers that comment on cytotoxicity (i.e., gamma-H2AX, phospho-histone H3, p53, and cleaved parp) and a constellation of biomarkers that comment on cytotoxicity (i.e., gamma-H2AX, phospho-histone H3, p53, and cleaved parp). In summary, each of the genotoxicants was correctly identified as such, and their genotoxic mode of action was evident from accompanying biomarker signatures. Furthermore, the results with brefeldin A suggest the system is able to highlight false positive micronucleus test results.

2816 Assessment of Chemical-Induced Mutagenesis in Mice and Rats Using Error-Corrected Next Generation Sequencing


Recent advances in Error Corrected-Next Generation Sequencing (EC-NGS) technology has reduced the sequencing error rate of standard Next Generation Sequencing (NGS) by 100,000-fold. The high error rate of standard NGS has prevented its use in Genetic Toxicology to assess mutagenicity of test articles in vivo, thus requiring use of transgenic rodent (TGR) mutation assays to assess in vivo mutagenesis. Big Blue® (BB) transgenic rodent (TGR) mutation assays, the gold-standard for measuring in vivo mutagenicity (OECD Test Guideline 488), can be used to assess the ability of DNA damage and repair pathways to modify the number of mutations per cell. The approach permits measurement of MF and mutations in any species, tissue, or DNA segment. The error rate of DS, below the spontaneous mutant frequency of mammalian genomic DNA, permits detection of both background and chemical-induced increases in MF. Here, we compared the MF and spectra for the traditional cl− cl+ gene plaque assay to DS in BB mice exposed to vehicle (olive oil), N-ethyl-N-nitrosourea and benz(a)pyrene. This bridging study showed plaque and EC-NGS methods yielded similar fold increases in MF and shifted in spectra demonstrating equivalence. We then evaluated four endogenous genes in BB mice covering 15 KB of genomic DNA. We then analyzed rats with similar exposures but used a 50 KB probe set covering 20 genomic sequences across chromosomess, RNU and RPS and produced similar large increases in MF over vehicle controls in both species. Unsupervised cluster analysis of simple mutual spectra and trinucleotide spectra showed that each treatment condition produced similar patterns in mice and rats that were unique for each treatment condition. The data show that EC-NGS is equivalent at detecting mutations in the cl− gene. Advantages are that EC-NGS is robust in detecting the MF in both MF and mutant spectra in endogenous DNA in two rodent species using both a gene-based probe set in mice and a representative genomic approach used in rats. In summary, our results show that an EC-NGS technology can measure in vivo mutagenesis associated with nonclinical biomarkers of human cancer risk.

2817 Modification of DNA Damage and Repair Pathways Reveals Detailed Mechanistic Information on the Genotoxicity of Clastogens

S. M. Bryce, J. C. Bemis, and S. D. Dertinger.

The miniaturized and automated in vitro assay platform, - MultiFlow - has demonstrated its utility for deriving genotoxic mode of action information. Recent adaptations of this approach yield additional levels of insight into aneugenic and clastogenic mechanisms. Here we report on the latest advancement that combines established biomarkers of genotoxicity with agents known to alter specific DNA damage and repair pathways. TK6 cells were preincubated with the following agents to modify their respective targets - olaparib a PARP inhibitor that affects base excision repair (BER), NU7441 a DNA-PK inhibitor that affects non-homologous end joining (NHEJ), MK8876 a CHK1 inhibitor, and finally a cocktail of reactive oxygen species (ROS) scavengers for investigating oxidative stress-mediated damage. Cell aliquots from each of these conditions were then exposed to genotoxic agents with varied clastogenic activities, i.e. the ROS generators hydrogen peroxide (H2O2) and menadione (Mna), a DNA synthesis inhibitor hydroxyurea (HU), campothecin (Cam) and doxorubicin (Dox) as topoisomerase I and II inhibitors respectively and methyl methanesulfonate (MMS) as an alkylator. Cells were collected at 4 and 24 hrs of exposure and examined for γH2AX and p53 responses. Dose-response data were converted to area under the curve (AUC) values. AUC values for Mna and H2O2 were dramatically reduced in the presence of ROS scavengers, while none of the other test articles showed such attenuation. Eto responses were potentiated by NU7441 consistent with the role of NHEJ in response to topo II poisons. HU effects were potentiated by MK8876 an agent known to modify DNA synthesis inhibition. Camp- and MMS-induced responses were potentiated with olaparib, highlighting the role of BER in response to these agents. Overall, these varied profiles of response potentiation and attenuation provided a means to differentiate between the multiple classes of clastogens. In addition to the AUC approach, we are investigating other data analysis approaches and assessing their performance. The use of specific modifiers of DNA repair pathways and ROS-mediated damage to the existing MultiFlow assay provides signatures of test article activity that can be used to investigate mechanisms and molecular targets. This information will be of great utility for risk assessment tools such as adverse outcome pathways and in vitro to in vivo extrapolation.

2818 High-Throughput Screening for Resistance to Colon Cancer-Associated Carcinogen in Budding Yeast Identifies Both DNA Repair and Ribosomal Protein Genes

M. Fasullo, M. Dolan, N. St. John, and F. Zaidi.

Risk factors for colon cancer include both diet and genetics; charred meat contains heterocyclic aromatic amines, such as 2-amino-3-methylimidazo(4,5-f)quinoline (IQ). However, few IQ resistance genes have been identified. We used yeast as a model organism to determine genetic susceptibility to IQ by introducing expression vectors that contain both human CYP1A2 and N-acetyl transferase (NAT2) genes into the yeast deletion library. To identify resistance genes, we used a high throughput approach for screening the “humanized” and the original yeast deletion library. Pooled cells from both libraries were exposed to 400 μM and 800 μM IQ, the DNA barcodes were sequenced using the Illumina platform, and statistical significance was determined for exactly matched barcodes. We identified 117 IQ resistance genes from the yeast library expressing human genes. Using EIXIR STRING database, we identified a highly interacting set of ribosomal protein genes, including RPL26A, RPL26B, RPL23, RPL33, RPL4A, RPS16B, and RPS6B. Upregulation of these genes are correlated with multidrug resistance while downregulation confers sensitivity to several cross-linking DNA damaging agents, including oxaclipatin. In a separate screen, DNA repair genes, RAD18 and NTG1, were identified. Interestingly, a low IQ resistance gene, RAD9, has not been documented to be risk factors for colon cancer and ntg1 knockout mice develop colon cancer. These studies thus suggest that activated IQ targets housekeeping genes involved in genome stability as well as protein synthesis. Future experiments are planned to knock-down human orthologues of the yeast genes and conduct similar screens in human cell lines.
tein expression of p-Tau, γ-synuclein, and presenilin 1 and 2 were assessed in brain tissue at 12 wk after WF exposure ended. Results suggest that WF inhalation increased telomere length without affecting telomerase in whole brain. Moreover, we observed that components of the shelterin complex, Trf1 and Trf2, play an important role in telomere end protection, and their regulation may be responsible for the increase in telomere length. In addition, expression of different neurodegeneration markers, such as p-Tau, presenilin 1-2 and γ-synuclein proteins, were increased in brain tissue from the WF-exposed rats as compared to control. These findings suggest a possible correlation between epigenetic modifications, telomere length alteration, and neurodegeneration because of the presence of factors in serum after WF exposure that may cause extra-pulmonary effects as well as the translocation of potentially neurotoxic metals associated with WF to the central nervous system (CNS). Further studies are needed to investigate the brain region specificity and temporal response of these effects.

### 2820 Duplex Sequencing Provides a Sensitive Method for Early Detection of Mutations Induced by Chemical Exposure in Vitro: A Concentration Response and Time Course Experiment in Human TK6 Cells Exposed to N-ethyl-N-nitrosourea


Duplex Sequencing (DuplexSeq), a form of error-corrected DNA sequencing, has an ultra-low error rate, allowing for detection of rare mutations within individual samples. This technology has wide-ranging applicability in diverse genomics fields, from paleo-genomics to forensics, and is especially useful in clinical applications such as early detection of diseases, e.g., cancer, and monitoring response to treatment over time. In a collaborative effort among the National Toxicology Program, TwinStrand Biosciences, and Integrated Laboratory Systems, the application of DuplexSeq to mutagenicity testing was evaluated in vitro. Human TK6 lymphoblastoid cells (TK6 cells) were exposed to N-ethyl-N-nitrosourea (ENU) at concentrations ranging from 25 - 200 micromolar at 24, 48, 72, or 96 hours. Cell samples collected at each time point were evaluated for mutation frequency, with statistical analysis using the Duplex Sequencing Human Mutagenesis Panel developed at TwinStrand Biosciences. Genotoxicity, measured by micronucleus frequency, was also evaluated in the same ENU-exposed TK6 cells to provide confirmation of ENU-induced genetic alterations. The performance of DuplexSeq was excellent in the TK6 cell samples, with a yield of over 1 billion duplex bases in 98% of the samples and high concordance between duplicates. As expected, MF increased with increasing concentrations of ENU and frequencies were higher at the later time points in samples exposed to the highest ENU concentrations. An increase in MF relative to vehicle control was observed even at the lowest concentration of ENU. The mutational spectrum did not vary with ENU concentration or exposure duration. Interestingly, there was a non-canonical increase in C to T mutations across all the samples; however, increased C to T substitutions following exposure to ENU is consistent with previous reports in both TK6 cells and in tissues collected at early timepoints (less than 96-hours) in ENU-exposed rats. These results indicate considerable potential for applying DuplexSeq to in vitro genetic toxicity testing.

### 2821 Scoring of the In Vitro Micronucleus Assay Using Imaging Flow Cytometry and Deep Learning

M. A. Rodrigues, B. Davidson, M. Riedel, Y. Li, and V. Venkatachalam.

The in vitro micronucleus (MN) assay is well-established for evaluating genotoxicity and cytotoxicity and is often scored by manual microscopy which is laborious and subject to low throughput and scorer variability. Automated scoring methods such as slide-scanning microscopy and flow cytometry have been developed but have drawbacks. Microscopy requires high quality slides to be created and flow cytometry lacks the ability to visually confirm the legitimacy of MN. The use of imaging flow cytometry (IFC) to perform the assay possesses the potential to overcome these limitations by combining the speed and statistical robustness of conventional flow cytometry with high-resolution imaging capabilities of microscopy. The use of IFC to perform the MN assay is demonstrated using several cell lines and a number of common genotoxicants. Cells are imaged in suspension, eliminating the requirement to create microscope slides, permitting imagery of thousands of key events to be captured in just a few seconds. Traditionally, the MN assay performed by IFC has relied on feature-based image analysis to identify key events and quantify MN. In this study, we developed a classification model using convolutional neural networks (CNNs) to score the MN assay. Image classification by CNNs presents several advantages in comparison to traditional feature-based scoring methods, including elimination of complex image analysis strategies as well as translatability across multiple cell lines and test compounds. Here we show that a single CNN-based classification model is able to score both the cytokinesis blocked and unblocked versions of the MN assay using TK6 lymphoblastoid cells and three widely used test chemicals: Mitomycin C, Etoposide and Mannitol. All samples were also scored by manual microscopy to evaluate feature-based algorithms, permitting direct comparison of all scoring methods. We demonstrate that CNN-based scoring is able to correctly identify statistically significant increases in MN frequency, outperforms feature-based methods and compares well to manual slide microscopy. The use of IFC and deep learning presents a further step towards a fully automated scoring solution for the MN assay. This approach introduces a number of novel elements and potential improvements towards overcoming many challenges inherent in conventional techniques.

### 2822 Next Generation Genotoxicity Assessment in Human Hepatocyte Models: CometChip and Micronucleus Assay in Metabolically Competent HepaRG Cells


We are developing medium throughput genotoxicity assays using human-relevant metabolically competent hepatocytes to reduce, replace and refine the use of animals in the practice of genetic toxicology. We are combining HepaRG™ cells with CometChip® technology, a single cell array platform developed at MIT, and flow cytometry-based micronucleus (MN) assay to develop a New Alternative Methodology (NAM) aimed at reducing reliance on the in vivo comet and micronucleus assay. CometChip® facilitates rapid processing of 96 samples with unbiased automated image-based scoring of the comet assay that can replace 30 yr old slide-by-slide one cell at a time scoring. Each imaged well of the 96 well plates have ≥ 200 scorables comets with the entire plate scored in less than 45 minutes compared to days needed to score 96 samples using traditional comet assay scoring. The in vitro and in vivo MN assay are part of the ICH S2R1 genetic toxicity test battery and we have adapted the flow cytometry-based micronucleus (MN) assay for use in HepaRG™ cells. To qualify this approach as a NAM we have developed an initial basic protocol using a 3-day repeat exposure regimen, established qRT-PCR assays for functional assessment of HepaRG™ metabolic competency, conducted "power" studies to determine optimal number of comets scored per each sample, trained external collaborators at ILS, completed testing of an initial “training set” of negative and positive control test articles for use in the qualifying the HepaRG™ CometChip® assay, and integrated HepaRG™/CometChip® assay with MN assay to follow up in vitro MN positive responses. Multiple endpoint genotoxicity assessments in human hepatocyte models can serve as alternatives to animals for equivalent or better human-relevant safety and risk assessments than relying solely on rodent models. This work is funded by NIEHS SBIR 4R44ES024698-02.

### 2823 Impact of DNA Polymerase ζ in the Mutation Signature of Methylating Agents


DNA Polymerase ζ (Pol ζ) is a low fidelity B-family polymerase involved in DNA damage tolerance by mediating replication after DNA adducts are formed from exposure to genotoxic chemicals. While it can be kinetically inefficient at inserting a base opposite DNA adducts, it is proficient in extending DNA from base modifications or mismatches, thus its expression and function can impact the mutation signature of methylating agents. This work is funded by NIEHS SBIR 4R44ES024698-02.

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### 2824 Impact of DNA Polymerase ζ in the Mutation Signature of Methylating Agents


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found in human cancer genomes. The objective of this study was to elucidate the role of DNA Pol ζ in mutagenesis arising from chemically induced DNA methylation. The experimental strategy involved exposing mouse embryonic fibroblast cells deficient in REV3 (Pol ζ catalytic subunit) to 1-methyl-3-nitro-1-nitrosoguanidine, an alkylating agent that induces O6-meG formation. Clonally expanded mutant variants were analyzed by whole-genome sequencing for patterns of mutations that could be used to establish a strategy for in vitro establishment of mutation signature analysis of chemically deficient in REV3-deficient cells, and an increase in overall number of mutations in the REV3-deficient cells. Focusing on variant calling analysis for each DNA triad sequence, mutation signatures were identified consisting of high G>A mutation frequency in cells deficient in REV3. These data suggest a previously unrecognized role for Pol ζ in reducing mutation frequency in mammalian cells exposed to methylating agents. Furthermore, the experimental approach and data analysis pipeline established in this work, involving mutation signature analysis of chemically exposed cell lines, provides an advanced genome-wide in vitro strategy for understanding the interplay of gene-environment interactions driving the mutagenicity of chemicals.

2824 Treatment of SEB-Induced ARDS with CBD Ameliorates Fatal Inflammatory Response
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The novel SARS-CoV-2 virus known to cause the COVID-19 outbreak has claimed the lives of over 240,000 Americans and 1,270,000 people worldwide, with cases consistently rising daily. Thus, understanding the mechanisms behind this disease are vital at this time. With the absence of appropriate research infrastructure to handle the virus and a refractoriness of rodents to this disease, Staphylococcus enterotoxin B (SEB)-induced Acute Respiratory Distress Syndrome (ARDS) model mimics the cytokine storm and fatality presented in patients with severe COVID-19. When C3H/HeJ mice were exposed to a dual dose of SEB, their survival dropped to 0% in ~5 days. In this study, we administered cannabidiol (CBD) intraperitoneally for 3 days pre- and post-SEB dosing and found that the survival rate increased to 100% indefinitely. Initial evaluation of scRNAseq data from lungs comparing naïve to SEB-induced ARDS mice illustrated an increase in neutrophils, inflammatory macrophages, and a loss in lung epithelial cells in the latter group. When evaluating the effect of CBD treatment on SEB-induced ARDS, we were able to demonstrate that CBD reduced the macrophage population. To characterize the mechanism by which CBD treatment ameliorated the inflammatory response, we found that CBD treated mice had significant reduction in TNF-α and IL-1β. The expression of these cytokines is directly associated with the presence and activation of inflammatory macrophages and neutrophils presented in ARDS. MicroRNA microarray differential expression analysis showed a significant increase in the expression of mmu-miR-124-3p, mmu-miR-21a-5p, and mmu-miR-140-5p with CBD treatment. Ingenuity Pathway Analysis (IPA) indicated that mmu-miR-21a-5p targets IL-1β, and mmu-miR-140-5p targets TNFα, while mmu-miR-124-3p targets both IL-1β and TNFα. The miRs were also important in pathways associated with respiratory disease and inflammation. This finding offers insights for the development of preventive and therapeutic strategies in the treatment of ARDS, including that induced in COVID-19. Supported by NIH grants P01AT003961, P20GM103641, R01ES030144, R01AI129788 and R01AI123947.

2825 Comparison of Overall Immune Status among Orchard, Greenhouse, and Open-Field Farmers
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Agricultural workers could be exposed to various work hazards including inorganic or organic dust, pesticides, malodorous compounds, or pathogenic microorganisms. The organic dust generated in livestock confinement buildings is known to cause a broad spectrum of respiratory illness. Since not much systemic investigations have been undertaken for immune status associated with respiratory hypersensitivity on orchard, greenhouse, or open-field farmers, this study aimed to compare various immunologic markers of these farmers with no significant differences in age and gender distribution (19 grape farm workers, 48 open-field onion farm workers, and 21 greenhouse rose farm workers). Blood taken from the farmers was subjected to plasma IgE, IgG1, IgG4, and IgA measurements. Isolated peripheral blood mononuclear cells (PBMC) were used for flow cytometric phenotyping of major immune cell subpopulation, and stimulated for measurement of cytokine production. The onion farmers have significantly higher plasma IgE (475±62 ng/ml) and lgG1 (6.0±0.4 mg/ml) levels, and lower IgA (3.0±0.2 mg/ml) levels than the rose farmers (IgE: 272±76 ng/ml, IgG1: 3.7±0.5 mg/ml, IgA: 3.9±0.4 mg/ml), proportion of cytotoxic T Lymphocyte was lower in the onion (17.2±2.2%) and the grape (18.0±1.0%) farmers than the rose farmers (20.3±1.7%). On the other hand, proportion of natural killer (NK) T cell was significantly higher in the onion (2.0±0.6%) and the grape (2.3±0.3%) farmers than the rose farmers (0.9±0.4%). NK-T cell subset may play a crucial role in its association with allergic diseases. NK cell proportion was significantly lowered in the grape farmers (19.8±3.3%) when compared with the other farm workers (onion farmers: 26.5±1.9%, rose farmers: 26.9±2.5%). Considering the skewedness toward type-2 helper T cell response, ratios of interleukin (IL)-4: interferon (IFN)-γ and IL-13:IFN-gamma were significantly higher in the grape workers than the other workers. Overall, among the three agricultural worker groups, the grape workers are more vulnerable to allergic immune alteration followed by the onion farmers. Supported by Korea Rural Development Agency Project No. PJ014296022020.

2826 Inhibition of Glycolysis Attenuates Excitation-Contraction Coupling in Human Airway Smooth Muscle Cells
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Obesity aggravates asthma by enhancing airway hyperresponsiveness (AhR) and attenuating response to standard asthma therapies. Dysfunctional metabolism in obesity leads to several obesity-related co-morbidities, including asthma. However, the mechanistic link between obesity and AhR remains poorly understood. Airway smooth muscle (ASM) cells are the predominant airway structural cells contributing to AhR and show amplified agonist-induced cytotoxic Ca2+ (Cyt-Ca2+), Myosin Light Chain (MLC) phosphorylation and force generation in obese individuals. Therefore, we hypothesized that altered metabolic profile in obesity amplifies excitation-contraction (EC) coupling in human ASM (HASM) cells. Lysates of HASM cells from age, gender, and race-matched obese and non-obese donors (n = 8 donor pairs) were screened with liquid chromatography-mass spectrometry to determine polar and non-polar metabolites. Seahorse assay was used to measure mitochondrial respiration and glycolysis rates in HASM cells. In metabolomic screening, obese donor-derived HASM cells showed a unique signature, characterized by changes in metabolites of glucose metabolism, including glycolysis and citric acid cycle. We determined that Cyt-Ca2+ in HASM cells were pre-treated with the phosphofructokinase inhibitor PKF155 (0.1 - 10 μM, 5 min -4h), and carbachol (CCh) or histamine-induced phosphorylation of MLCK, MLCP and phosphatase and protein kinase B (Akt) were determined. Agonist-induced cytotoxic Ca2+ and Isoproterenol-induced cyclic AMP MPPD were measured after PKF155 (10 μM) pre-treatment. The glycolysis inhibitor PKF155 attenuated CCh or histamine-induced MLC phosphorylation in a concentration (CCh: 0.16 ± 0.038 of control, n = 5 donors; histamine: 0.04 ± 0.017 of control, n = 6 donors) and time (CCh: 0.22 ± 0.063 of control, n = 6 donors)-dependent manner. Meanwhile, phosphorylation of Akt (0.175 ± 0.040 of vehicle control, n = 6 donors) and Myosin Light Chain (MLC) (0.26 ± 0.09 of control, n = 6 donors) were attenuated by PKF155 pre-treatment. These cellular outcomes were unassociated with changes in agonist-evoked Ca2+ mobilization or cyclic AMP production. Further, in human Precision-Cut Human Lung Slices (hPCLS), PKF155 pre-treatment (10 μM, 10 min) attenuated CCh-induced bronchoconstriction (AUC: 0.39 ± 0.02 mg/ml) and a loss in lung epithelial cells in the latter group. When evaluating the expression of these cytokines is directly associated with the presence and activation of inflammatory macrophages and neutrophils presented in ARDS. MicroRNA microarray differential expression analysis showed a significant increase in the expression of mmu-miR-124-3p, mmu-miR-21a-5p, and mmu-miR-140-5p with CBD treatment. Ingenuity Pathway Analysis (IPA) indicated that mmu-miR-21a-5p targets IL-1β, and mmu-miR-140-5p targets TNFα, while mmu-miR-124-3p targets both IL-1β and TNFα. The miRs were also important in pathways associated with respiratory disease and inflammation. This finding offers insights for the development of preventive and therapeutic strategies in the treatment of ARDS, including that induced in COVID-19. Supported by NIH grants P01AT003961, P20GM103641, R01ES030144, R01AI129788 and R01AI123947.

2827 Single Cell Profile of LPS-Induced Acute Respiratory Distress Syndrome Shows an Increase of Reg3g, Scgb1a1, and Scgb3a Expression with i3C Treatment
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Acute lung injury and acute respiratory distress syndrome (ALI/ARDS) are clinical disorders that manifest from the inflammatory response in the lungs due to direct and indirect activation of the immune cells. The aggressive reaction of immune cells directly impacts disease severity, leading to alveolar damage, breakdown in the lung epithelial cell barrier, and impaired pulmonary functioning. Interestingly, the aryl hydrocarbon receptor has been shown to regulate Th22 and lung progenitor cell differentiation, which play a crucial role in reparative re-epithelialization and lung maintenance. Our studies aim to determine if indole-3-carbinol, a naturally occurring AhR ligand, promotes...
accurate barrier reconstitution and restores lung functioning through dif-
ferential regulation of epithelial genes and immune-mediated mechanisms. Towards this, 5mg/kg of LPS was intranasally administered in C57BL/6 mice to induce ALI, and the mice were treated with 80mg/kg 13C i.p. three hours after disease induction. After 48 hours, mice lung compliance was tested using Buxco plethysmography, and it was noticed that LPS + 13C treated-mice have similar basal functionality to naive mice. Histopathological analysis revealed that 13C prevented atelectasis and poorly organized epithelium repair in LPS-administered mice. Furthermore, 13C increased the population of Th22 cells and increased the expression of IL-22 in bronchoalveolar lavage fluid, which are mediators of re-epithelialization and antimicrobial peptide pro-
duction. Single cell RNA sequencing of the instilled 13C-stained mouse showed upregulation of Scgb1a1, Scgb3a1, and Reg3g genes expressed by club and nonciliated secretory cells, variant Clara cells, and lung progenitor cells. IL-22 regulates Reg3g. In conclusion, our studies suggest that 13C alleviates lung injury and impaired pulmonary compliance by restoring lung epithelium, which may be mediated by IL-22 and epithelial-associated genes Reg3g, Scgb1a1, and Scgb3a.

2828 Next Generation Risk Assessment Approach for Inhalation: Polymer Case Studies
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Next Generation Risk Assessment (NGRA) is an exposure-led, hypothe-
sis-driven approach that integrates new approach methodologies (NAMs) to assure human safety without animal data. We are currently evaluating a NGRA approach for inhalation exposures using hypothetical case studies of a polymer in personal care products (e.g. antiperspirants) and a slanie in cleaning products. Impairment of mucociliary clearance, lung fibrosis and lung surfactant inhibition were identified as the relevant endpoints for the most common consumer exposure scenarios (e.g. daily use of an anti-
perspirant). To investigate these endpoints, two cell models were selected for in vitro testing: the MuclAir™-HF cell model (Epithelix) a system which shows ciliated as well as mucous producing cells typical for the bronchial region and the EpiAlveolar™ cell model (MatTek) a coculture system out of AT1 and AT2 (surfactant producing) cells, fibroblasts and THP1 cell represent-
ing the most common cells of the alveolar tract. In addition to the two case study chemicals another 16 benchmark substances were selected either due to their well-known effects in the specific areas of the lung, history of safe use and/or due to chemical or physical similarities to the case study chemicals. Linking the in vitro point of departures to the relevant in vivo consumer ex-
posure level is essential to evaluate the usefulness of the in vitro test systems. Therefore, consumer habits and practices were used to derive an airborne concentration (mg/m³) for each chemical and exposure scenario, which was then transformed into deposited mass in the bronchial and alveolar region (µg/cm²) using MPPDv2.8. Cells were exposed daily for up to 12 days in vitro and different endpoints measured every other day. Preliminary results indi-
cate that the most severe transition in the cells along with barrier disruption over time as evident from the increased alveolar cross-sectional area along with decreased ZO1 (Zonula Occludens 1) expression and decreased TEER measurement. In summary, our findings demonstrate the relevance of reproducing key physiological condi-
tions such as cyclic stretch and air-liquid interface for in vitro toxicity studies. Together with the potential for including patient-derived cell complexities, this lung-on-chip is a promising alternative tool to animal-based inhalation toxicity studies.

2830 Utilization of Human Evidence for Testing and Assessment of Chemical Respiratory Sensitizers
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Despite high regulatory need, there remains a lack of internationally har-
monized approaches to identify chemical respiratory allergens. An Adverse Outcome Pathway has been outlined to identify hazard identification ap-
proaches to assess chemicals for this endpoint. In order to evaluate in vitro and in silico approaches, a reference list of 120 putative respiratory sensitizer-
s was generated based on structural alerts. In this abstract, we discuss the methodology for utilization and curation of human data towards validating the reference list of putative respiratory sensizers. This approach utilized the Bioaffects database and the NCEA Guidance, and the NGRA approach provides a standardized method to evaluate and apply human clinical data as part of the weight-of-evidence towards establishing reference chem-
ical lists. The output can be used along with other available data and chemi-
cal characteristics to establish an internationally harmonized reference list of chemicals, including respiratory sensitizers, irritants, and non-sensitizers, to update existing risk assessment approaches and evaluate new approaches for this key endpoint.

2829 Toxicity Profiling of Compounds and Nanoparticles in a Breathing Lung-on-Chip Model
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Inhalation is a major route for exposure where the pulmonary epithelium serves as the portal of entry to the systemic circuit for the inhaled airborne toxics. This leads to persistent and progressive inflammation of the alve-
olar epithelium which is a key event in the pathogenesis of various chronic lung diseases like emphysema and lung fibrosis. Inhalation exposure tests in animals present species-specific variations and makes it difficult to draw conclusions in human. Hence, current research efforts have been focused to develop alternative, biologically-relevant in vitro models. In this quest, we have utilized microfluidic devices to recreate a breathing alveolar in vitro model to study toxicity. To this end, we have used human lung alveolar ep-
thelial cells with the AXLung-on-Chip System (AlveoliX). We subjected the
cells to cyclical stretch when they demonstrated an intact barrier. To develop physiologically relevant models, we additionally optimized air-liquid interface (ALI) on the chip. As a proof of concept, to recreate occupational inhalation of nanoparticles and toxic compounds, we exposed the lung epithelial barrier to varying doses of ZnO nanoparticles and PHMG (CAS 89697-78-9) using the Vitrocell cloud-12 exposure system. Additionally, we instilled cells with TGFβ1 to mimic fibrosis related injury on the chip. Our results demonstrated a stable barrier formation in the cells over time represented by distinct tight junction protein expression and gradual increase in transepithelial electrical resistance (TEER). Cyclical stretch was shown to increase barrier permeability, but the cells were able to adapt to this stress over time. Aerosolized ZnO nanoparticle
causés significant increase in toxicity in stretched cells compared to cells in static conditions. Furthermore, decreased barrier integrity was ob-
served with PHMG exposure with cells in cyclical stretch and ALI conditions with respects to cells in static and submerged conditions respectively. TGFβ1 treatment in submerged conditions triggered an epithelial-mesenchymal transition in the cells along with barrier disruption as evident from the increased alveolar cross-sectional area along with decreased ZO1 (Zonula Occludens 1) expression and decreased TEER measurement. In summary, our findings demonstrate the relevance of reproducing key physiological condi-
tions such as cyclic stretch and air-liquid interface for in vitro toxicity studies.

2831 Alveolar Macrophage Phenotype Contributes to Sex Differences in Nanoparticle-Induced Lung Disease
J. L. Ray, and A. Holian. University of Montana, Missoula, MT.

The global respiratory health crisis is well-established: ~1 billion people are affected, with 4 million deaths annually due to chronic disease. However, much of this disease burden could be mitigated by reducing exposure to environmental and occupational air pollution, especially in sensitive pop-
ulations. Lung diseases of both sexes are affected with a unique etiology and/or severity and those associated with eosinophilic inflammation (e.g. allergic asthma) are widely reported to have a female-bias. However, research on the
exact mechanisms and responsible cell types remains inconclusive. Moreover, these mechanisms have yet to be explored in the context of non-allergic eosinophilia following particle exposure. Recently, we reported that a female bias also occurs in a model of nanoparticle-induced non-allergic eosinophilia: female mice develop more severe eosinophilic airway inflammation following multi-walled carbon nanotube (MWCNT) exposure compared to males. The importance of alveolar macrophages (AMs) in regulating the respiratory immune response is well-known and AMs are considered to be the primary cell type responsible for responding to inhaled nanoparticles. AMs are highly plastic cells with the ability to adopt a spectrum of functional phenotypes; based on the observed eosinophilia, we hypothesize that AMs primarily develop a M2a phenotype following MWCNT-exposure causing a NO-dependent type II inflammatory signaling and eosinophil recruitment. Therefore, the present research sought to investigate the hypothesis that sex-differences in AM M2a phenotype development are responsible for the female-bias in MWCNT-induced eosinophilic inflammation. The functional phenotype of AMs from MWCNT-exposed male and female mice was determined by measurement of cytokine production, gene expression, and transfection factor activation. Results demonstrated greater production of M2a-associated cytokines and mRNA, and increased activation of the M2a phenotype-associated IL-4/13-STAT6 pathway in AMs from female mice compared to males. There is also evidence that hormone signaling may play a role in the activation of M2a-associated pathways. Taken together, these results further our limited knowledge of the mechanisms responsible for sex-biases in particle-induced lung inflammation and the role of AMs in associated disease pathogenesis. Supported by R21 ES030978 and P30 GM103338.

2832 Single Cell RNA Sequencing Identifies Multiple Genes Related to T Regulatory Cells Induced in Cannabinoid Treated Staphylococcus Enterotoxin B (SEB)-Induced Acute Respiratory Distress Syndrome (ARDS)

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COVID19/ARDS has been declared by World Health Organization (WHO) as an outbreak and significant threat to the international health. ARDS has claimed deaths of 250,000 deaths in USA and 1.3 million globally while more than 11 million have been infected in USA and 54 million globally. There is no specific effective treatment until this moment. In this study, we used a single dose of Staphylococcal enterotoxin B (SEB) (50ug) intra-nasally which acts as a superantigen to induce Acute Respiratory Distress Syndrome (ARDS). The inhalation of SEB, which is a category B agent of bioterrorism as defined by CDC, leads to robust activation of T cells and a cytokine storm that causes significant damage to the lungs. In the current study, we induced SEB-mediated ARDS in C57BL/6 mice and investigated if treatment with anandamide (AEA), an endocannabinoid would attenuate ARDS. Our data demonstrated that a dose of (40mg/kg) of AEA significantly improved the clinical parameters including lung function tests in mice with SEB-induced ARDS when compared to SEB+VEH controls, as determined by plethysmography. Analysis of single cell RNA sequencing (scRNAseq) of lungs of SEB+AEA mice showed significant increase in genes related to T regulatory cells and their subsets such as CD4, CCL4, MAF, GATA3, IKZF2, CTLA4, IL2-R, NL3G and SELL when compared to SEB+VEH. The results have been confirmed by RT-PCR. Additionally, our flow cytometry data demonstrated significant induction of markers related to T reg population when compared to SEB+VEH. Pro-inflammatory cytokine storm is considered one of the outcomes of ARDS. Our ELISA data indicated that pro-inflammatory cytokines such as TNFa and IL6 in Bronchoalveolar lavage fluid (BALF) of SEB+AEA mice significantly decreased compared to SEB+VEH. Together, our scRNASeq data demonstrated that the endocannabinoid, AEA attenuated ARDS and inflammation mediated by SEB by improving the functional properties of lung tissue resident T regulatory cells which mediated the suppression of T cell activation and decreased proinflammatory cytokine storm. Supported by NIH P01AT003961, P20GM103641, R01AI217988, R01 ES031044 and R01AI123947.

2833 Benchmark Dose Modeling Approaches for Volatile Organic Chemical Exposure to Human Airway Epithelial Cells at Air-Liquid Interface


Currently, volatile organic chemicals (VOCs) use a unique problem for traditional in vitro chemical safety testing which is predominantly performed in high-throughput subacute exposures. Most VOCs are insoluble in water and inhalation is the most concerning route of exposure. To address the difficulties in screening toxic effects of VOCs, the cell culture exposure system (CCES) permits cells to be exposed to multiple concentrations at air-liquid interface (ALI) in a 24-well format. ALI exposure methods permit direct pollutant-to-cell interaction with the test article at physiological conditions, providing a more realistic exposure paradigm. In the on-going study, BEAS-2B and 16HBE cell lines, as well as primary human bronchial epithelial (HBE) and MatTek Epi-Airway™ cells are used to assess acute and sub-acute responses to a variety of volatile chemicals. 1-bromopropane, 1,3-butadiene, carbon tetrachloride, dichloromethane, acrolein, acetaldehyde, trichloroethylene, and formaldehyde have been screened using BEAS-2B and HBE cells. The results show that CCES exposures of in-vitro cell lines exhibit variable toxicity across cell types. Cell viability and cytotoxicity are measured using the CellTiter-Glo assay and lactate dehydrogenase release. For most chemicals tested, the highest concentration shows between a 10-30% change in BEAS2B cell viability while contrasting, primary HBE cells show less cytotoxicity when exposed to almost all chemicals tested. Cell lyses were collected for TempO-Seq™ analysis designed to be used with benchmark dose (BMD) modeling response and comparison across cell types. The BMD for the most sensitive gene collection was achieved for all B2B cells exposed except for formaldehyde. Most striking is the comparison of BMD to threshold limit value (TLV) and results from existing in-vivo studies. Overall, the BMD measured for our chemicals was within one order of TLV magnitude reported by ACGIH and our targeted dose response reflects the same rank order of chemical potency as the relevant rodent in vivo exposures reported in the literature. Additional studies in comparison with Mattek™ Epi-Airway cells have been conducted for comparison. Overall, our study provides novel approaches to evaluate the capability of the transcriptomic data to identify concentration-dependent changes in gene expression for volatile chemicals and provides a jumping off point to evaluate the ability of the transcriptomic data to group chemicals with similar bioactivity profiles for potential read across applications. Abstract does not reflect views or policies of the US EPA.
Risk assessment and management relies on approaches that can accurately and efficiently predict the toxicity of chemicals in humans. Inhalation is a major route by which exposure to substances can occur, and is an area where resources have been dedicated to optimize human-relevant in vitro approaches. In this study, the INSPIRE Initiative (in vitro System to Predict Respiration toxicity), a two-dimensional (2D) human bronchial epithelial cell line (BEAS-2B) and a three-dimensional (3D) human reconstructed tissue model (MucAirTM; Epithelix) were used to predict the ability of chemicals to cause portal-of-entry effects on the human respiratory tract. The human cell-based systems were exposed to different concentrations of silanes (triethoxysilane (TES) and trimethoxysilane (TMS)) using a capillary dosage method and surfactants (Triton X-100 and/or oleoyl sarcosine) using atomization, at the air-liquid interface in a VITROCELL 6/4 exposure module. Nitrogen dioxide (NO2) was included as a positive control and sodium chloride and clean air (CA) or nitrogen gas (N2) as negative controls. Endpoints assessed included cell viability (ProtoplastTM assay), cytotoxicity (lactate dehydrogenase assay; LDH), and expression of inflammatory markers (interleukin-6, tumor necrosis factor-alpha) for the 3D tissues, morphology (hematoxylin and eosin staining), barrier integrity (transepithelial electrical resistance), and cilia beat frequency (SAVA system) were also examined. Preliminary studies demonstrated a concentration-dependent decrease in cell viability and an increase in cytotoxicity after 1 hour exposure of BEAS-2B cells to TES (0.72ppm, 25ppm, and 85ppm) as compared to CA. A significant increase in expression of inflammatory markers (interleukin-IL-6, IL-8, IL-2, and tumor necrosis factor alpha) was observed at 25ppm of TES. Studies are underway to assess additional test chemicals and endpoints in both systems. The results of this project can be used to better understand the usefulness of different test systems and, therefore, help guide selection. The results can also be used to predict the likelihood of a chemical to cause portal-of-entry effects on the human respiratory tract and inform regulatory decision-making.

Once weekly dosing rather than daily dosing is becoming increasingly common for respiratory studies to assess either safety or efficacy. This assessment using head-only plethysmography (HOP) was undertaken to better understand the effect of respiratory parameters with this regime. 4 mice were restrained on 4 occasions (Days 0, 7, 14, and 21) with data on tidal volume (TV), respiration rate (RR) and Respiratory minute volume (RMV) being captured after consuming a Western diet (WD) for 16 wk. For this study, 6 wk old male F334 rats developed metabolic dysfunction (MetDys) and expression of inflammatory markers (electrocardiography) were measured using EMMS data acquisition software. The data presented is the average for each of these timepoints. The RR rates ranged between 279 and 351 breathes/min with a mean value of 314 breathes/min and standard deviation (SD) of 26.5. The TV rates ranged between 0.141 and 0.233 mL/min with a mean value of 0.174 mL/min and SD of 0.0247. The RMV rates ranged between 40.1 and 78.2 mL/min with a mean value of 56.5 mL/min and SD of 9.0. Reviewing the RMV, TV and RR data for Days 0 and 21 for each of the 4 mice and compensating for bodyweight gave a mean decrease in RR/BW of 18%, TV/BW of 6% and RMV/BW of 11%. The RR/BW data for Days 7 and 14 were in accordance with this decrease (7% and 14% respectively). The RR/BW and RMV/BW were used to data variability. The RMV data in this assessment was compared against the Alexander equation1 over the same BW range (20.8 and 27.1 mL/min) but gave values that were significantly lower (by >5%) suggesting that species-specific RMV equation is necessary for delivered dose quantification on inhalation studies. The data suggests that the need to incorporate data from the restraint procedure when only restrained once per week and that the Alexander equation significantly underestimates the RMV in mice. Any further conclusions requires additional data to be collected due to the variability observed. 1 Alexander DJ et al. (2008). Inhal. Tox., 20, 1179-1189.

Isoprene hydroxy hydroperoxide (ISOPOOH) leads to the formation of secondary organic aerosols (SOA) generated from the reaction of vapor phase isoprene with atmospheric hydroxyl radical. This reaction is favored as anthropogenic air pollution levels have decreased in the atmosphere, limiting freely available NOx. Relatively little is known about the contribution of ISOPOOH in adverse human health effects from exposure to air pollution. However, previous studies have shown isoprene-derived SOA can induce inflammatory and oxidative gene expression in human airway epithelial cells (HAEc) and can lead to Nrf-2 activation. The objective of this study is to characterize the early mechanisms of oxidative stress induced by exposure of HAEc to non-cytotoxic concentrations of ISOPOOH. Our experimental approach relies on live-cell imaging of HAEc expressing roGFP, a genetically encoded fluorogenic sensor that specifically reports on changes in the glutathione redox potential (E_GSH) dynamically. Exposure to micromolar concentrations of ISOPOOH induced glutathione oxidation in HAEc through a mechanism that is independent of the generation of extracellular and intracellular H2O2, suggesting lipid peroxidation of the plasma membrane and/or oxidation of glutathione through the involvement of glutathione peroxidases. Supplementation of HAEc with selenium potentiated the effect of ISOPOOH on E_GSH, which implies glutathione peroxidase activity in transducing the peroxidative tone present by ISOPOOH to the cellular level. Inhibition of glutaredoxin prior to ISOPOOH exposure completely ablated the E_GSH response, demonstrating that roGFP is not directly oxidized but rather properly reports on ISOPOOH-induced E_GSH changes. These findings show that ISOPOOH is a potent and unique environmental oxidant that likely contributes to the oxidative burden posed by exposure to SOA. This abstract of a proposed presentation does not necessarily reflect US EPA policy.

Once weekly dosing rather than daily dosing is becoming increasingly common for respiratory studies to assess either safety or efficacy. This assessment using head-only plethysmography (HOP) was undertaken to better understand the effect of respiratory parameters with this regime. 4 mice were restrained on 4 occasions (Days 0, 7, 14, and 21) with data on tidal volume (TV), respiration rate (RR) and Respiratory minute volume (RMV) being captured after consuming a Western diet (WD) for 16 wk. For this study, 6 wk old male F334 rats developed metabolic dysfunction (MetDys) and expression of inflammatory markers (electrocardiography) were measured using EMMS data acquisition software. The data presented is the average for each of these timepoints. The RR rates ranged between 279 and 351 breathes/min with a mean value of 314 breathes/min and standard deviation (SD) of 26.5. The TV rates ranged between 0.141 and 0.233 mL/min with a mean value of 0.174 mL/min and SD of 0.0247. The RMV rates ranged between 40.1 and 78.2 mL/min with a mean value of 56.5 mL/min and SD of 9.0. Reviewing the RMV, TV and RR data for Days 0 and 21 for each of the 4 mice and compensating for bodyweight gave a mean decrease in RR/BW of 18%, TV/BW of 6% and RMV/BW of 11%. The RR/BW data for Days 7 and 14 were in accordance with this decrease (7% and 14% respectively). The RR/BW and RMV/BW were used to data variability. The RMV data in this assessment was compared against the Alexander equation1 over the same BW range (20.8 and 27.1 mL/min) but gave values that were significantly lower (by >5%) suggesting that species-specific RMV equation is necessary for delivered dose quantification on inhalation studies. The data suggests that the need to incorporate data from the restraint procedure when only restrained once per week and that the Alexander equation significantly underestimates the RMV in mice. Any further conclusions requires additional data to be collected due to the variability observed. 1 Alexander DJ et al. (2008). Inhal. Tox., 20, 1179-1189.

Oblesity affects over 30% of adults in the United and leads to metabolic changes in adipose function also known as metabolic dysfunction (MetDys). MetDys is a risk factor for lung function impairment, pulmonary hypertension and asthma. Inhalation of respirable crystalline silica causes significant respiratory morbidity and mortality in exposed workers due to silicosis. Silicosis is an incurable restrictive lung disease associated with inflammation and fibrosis. The question addressed by this study is, does pre-existing metabolic dysfunction increase a worker’s risk to pulmonary inflammation and silicosis? In previous studies, F334 rats developed metabolic dysfunction (MetDys) (weight gain, dyslipidemia, altered adipose function, and insulin resistance) after consuming a Western diet (WD) for 16 wk. For this study, 6 wk old male F344 rats were fed either a WD (40.6% fat (19.5%) lard, 40.6% total carbohydrate (20% sucrose), 14.8% protein) or standard rat chow (STD) (6.2% fat, 44.2% carbohydrate (grain sources), 18.6% protein) for 16 weeks before inhalation exposure to respirable crystalline silica (Min-U-Sil 5, 15 mg/m3) or filtered air (CA) or nitrogen gas (N) as negative controls. Endpoints assessed include cell viability (Prestoblue™ assay), cytotoxicity (lactate dehydrogenase assay; LDH), and expression of inflammatory markers (interleukin-6, tumor necrosis factor alpha) for the 3D tissues, morphology (hematoxylin and eosin staining), barrier integrity (transepithelial electrical resistance), and cilia beat frequency (SAVA system) were also examined. Preliminary studies demonstrated a concentration-dependent decrease in cell viability and an increase in cytotoxicity after 1 hour exposure of BEAS-2B cells to TES (0.72ppm, 25ppm, and 85ppm) as compared to CA. A significant increase in expression of inflammatory markers (interleukin-IL-6, IL-8, IL-2, and tumor necrosis factor alpha) was observed at 25ppm of TES. Studies are underway to assess additional test chemicals and endpoints in both systems. The results of this project can be used to better understand the usefulness of different test systems and, therefore, help guide selection. The results can also be used to predict the likelihood of a chemical to cause portal-of-entry effects on the human respiratory tract and inform regulatory decision-making.
Broblast differentiation and pulmonary fibrosis. Pam was cytotoxic to the primary human lung fibroblast cultures. Future work fibrotic diagnosis, co-treatment of 1-100μM diazepam inhibited expression primary human lung fibroblasts derived from donors with non-fibrotic and brotic cellular phenotypes in airway smooth muscle, we hypothesized that di-virtual 2021 SOT Annual Meeting and ToxExpo-stimulate OGR1 and activate distinct intracellular signaling pathways, Gαq and exploited other small molecules that have been reported to stimulate OGR1.~ in vitro-speak diverse G-protein signaling in response to changes in extracellular pH or sig-male and pulmonary airways lavaged to collect fluids (NALF and BALF). At the highest concentration over a 30 min period for both chemicals, minute vol-um and peak inspiratory flow began to decrease (males > females) indicat-ing airway irritancy. Acrolein but not TCE exposure led to significant increases in NALF lactate dehydrogenase activity and cellular inflammation (males ≥ females). Nasal and pulmonary inflammation was associated with marked depletion of circulating lymphocytes, monocytes, and neutrophils (each ~50% in case of acrolein) in both sexes (acrolein > TCE) indicating systemic effects, likely mediated by neuroendocrine activation as observed with ozone. Acrolein exposure also led to significant increases in NALF but not BALF TNF-α and CXCL1 (males > females). Acrolein increased IL-6 levels in both NALF and BALF (NALF > BALF) in both sexes; effect in NALF was greater in males when compared to females, indicating overall significant impact of acrolein in nasal airways whereas IL-6 levels are more affected in summary. Show that two selec-ted HAPs produced toxicity in nasal and respiratory airways that is linked to systemic depletion of white blood cell populations. When completed, this assessment will validate and refine on-going in vitro approaches for toxicity testing. This abstract does not reflect US EPA policy.

Targeting the Proton-Sensing GPCR OGR1 (GPR68) to Inhibit Pulmonary Fibrosis

Fibrosis is characterized as an aberrant wound healing response in which ex-cessive deposition of extracellular matrix proteins (ECM) destroy tissue archi-etecture and negatively impact organ function. In the lung fibrosis can result from environmental exposures, as a side effect of radiation treatment, or can develop in an idiopathic nature. Underlying these unique initiating events is a common effector, the fibroblast. Fibroblasts normally regulate extracellular-matrix deposition and maintain tissue structure. In a wound healing re-sponse, increased production and activation of the cytokine TGF-β1 leads to myofibroblast differentiation. Myofibroblasts secrete increased levels of extracellular matrix proteins such as collagen, and express markers such as alpha-smooth muscle actin (αSMA). In lung fibrosis, TGF-β1 is elevated and drives incessant differentiation of fibroblasts. Our lab focuses on the proton-sensing G-protein coupled receptor OGR1 (GPR68) in the context of myofi-broblast differentiation and pulmonary fibrosis. We have demonstrated that overexpression of OGR1 or stimulation with the small molecule Ogerin inhibits TGF-β1 induced myofibroblast differentiation. OGR1 is reported to stimu-late diverse G-protein signaling in response to changes in extracellular pH or in the presence of small molecules. To better understand the intracellular sig-naling responsible for the inhibition of myofibroblast differentiation, we have explored other small molecules that have been reported to stimulate OGR1. Benzodiazepines, including lorazepam and diazepam, have been reported to stimulate OGR1 and activate distinct intracellular signaling pathways, Gq, and Gαq respectively. Because the Gq pathway has been reported to inhibit pro-fi-brotic cellular phenotypes in airway smooth muscle, we hypothesized that dia-zepam but not lorazepam would inhibit myofibroblast differentiation. Using primary human lung fibroblasts derived from donors with non-fibrotic and fibrotic diagnosis, co-treatment of 1-100μM diazepam inhibited expression of αSMA and secreted collagen stimulated by 1ng/mL TGF-β1. 100μM loraze-pam was cytotoxic to the primary human lung fibroblast cultures. Future work to identify the mechanism through which these compounds are inhibiting TGF-β induced will enhance our understanding of the role of OGR1 in myofi-broblast differentiation and pulmonary fibrosis.

Paraquat (PQ) is an agrochemical known to cause pulmonary fibrosis. Paraquat-induced collagen deposition in the lung is thought to involve the initial formation of paraquat radicals by cellular diaphorases; but the spe-cific enzymes responsible for this bioactivating event in vivo have not been identified. The aim of this study was to test the hypothesis that lung P450 oxidoreductase (POR or CPR) plays a vital role in PQ-induced lung fibrosis in mice. A lung-Cpr-null mouse model was utilized, which und-ergoes doxycyline-induced, Cre recombinase-mediated deletion of the Cpr gene specifically in airway Club cells and alveolar type 2 cells in the lung. The lungs of lung-Cpr-null mice and their wild-type control littermates, male and female, were collected on day 14 after a single intraperitoneal injection of PQ at 20 mg/kg. Lung tissue sections were stained with picrosirius red for detection of collagen fibrils. The lung areas occupied by collagen fibrils were significantly larger (p<0.001) in PQ-treated wild-type mice, compared to sa-line-treated control groups. For lung-Cpr-null mice, the lung areas occupied by collagen fibrils were significantly smaller (by ~1.4-1.6 fold) than in sex-matched wild-type controls following PQ administration. The levels of mature collagen in lung tissue homogenate were also measured colorimetricaly, and found to be 1.7-1.9-fold lower (p<0.05) in PQ-treated lung-Cpr-null mice compared to wild-type mice. A sex difference was not detected in saline- or PQ-treated groups. These results indicate that lung POR plays an important role in PQ-induced pulmonary fibrosis. Supported in part by grants CA092596 and ES020867.
In vitro chemical risk assessment using human cells is emerging as an alternative to in vivo animal testing with reduced costs, fewer animal welfare concerns, and the possibility of greater human health relevance. In vitro inhalation toxicity testing of volatile compounds poses particular challenges. Here we report our efforts to establish a testing protocol in our own lab using the EpiAirway bronchial epithelium culture model and the VitroCell 12/12 system for air-liquid interface (ALI) exposures. For purposes of method development, we used methyl iodide (MeI) as a test compound. We examined viability, cytotoxicity, and epithelial integrity responses. Dose-dependent responses were observed with all assays. EpiAirway and BEAS-2B cytotoxicity responses to acute exposure were roughly similar, but EpiAirway was more resistant than BEAS-2B by the viability measurement, suggesting a proliferative response at low Mel concentrations. If wells were sealed to prevent evaporation, in-solution Mel concentration-response could be used to predict the response to Mel vapor within 2-fold by converting from the media- to the-air concentration at equilibrium using the blood:aair partition coefficient for Mel. The long-term stability of EpiAirway cultures enabled repeated exposures over a 5-d period, which produced responses at lower concentrations than did acute exposure. We are now using these in vitro methods to expose multiple cell culture models (eg, MuCell, AirSmart, AirEp, AlveoLuor) to 1,3-dichloropropene vapor, in order to determine tissue-specific local points of departure (PoDs). Airway dosimetry modeling will be used to in vivo equivalent external concentrations for these PoDs, which can be directly compared to empirically determined values from in vivo studies.

**2846 Inhalation Exposure of Acrylonitrile Butadiene Styrene Filament 3D Printer Emissions Induces Pulmonary and Systemic Toxicity in Rats**


Fused filament fabrication (FFF) 3-D printing is an emerging technology that has recently gained wide popularity among both consumers and manufacturers due to their increased product efficiency, reduced waste, and greater design flexibility. Numerous studies demonstrate that during thermal decomposition of the filament, incidental ultrafine particles (UFP) and volatile organic compounds (VOCs) with potential adverse respiratory health effects are released into the air. This study sought to evaluate the respiratory and systemic toxicity of emissions from printing with acrylonitrile-butadiene-styrene (ABS), the most common thermoplastic filament on the market. A representative dose concentration was used. 3-D printing generated particulates with average particle mass concentration of 240 ± 90 µg/m³, and an average geometric mean particle mobility diameter of 85 nm (geometric standard deviation 1.6). The number of macrophages in bronchoalveolar lavage increased significantly at day 15. IFN-γ and IL-10 were found among the treatments. There was an increase in platelets and monocytes in the circulation at day 15. Several serum biomarkers of hepatic and kidney functions were significantly higher at day 1. Under the current conditions of the experiment, it was concluded that the emissions from ABS filament caused minimal and transient pulmonary and systemic toxicity. This work is critical to fill the knowledge gap regarding the potential toxicological effects of exposure to the FFF 3-D emissions, which would help to establish effective control strategies and exposure limits to prevent adverse health effects from 3-D printing emission exposure.

**2847 Derivation of an Occupational Exposure Limit for β-glucans**

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β-Glucans are abundant bacterial, yeast, and fungal cell wall polysaccharides that have been shown to activate the immune system. Detectable concentrations of β-glucans have been identified in common occupational inhalation exposure scenarios associated with industries such as agriculture, food processing, and waste management. No exposure threshold values for inhalation of β-glucans have been established. To address this need, we derived a provisional occupational exposure limit (OEL) for β-glucan exposure based on a series of in vitro and in vivo studies. In vitro studies were performed in three organotypic models (Vitrocell 12/12 human bronchial epithelium, EpiAirway bronchial epithelium cell culture model and the VitroCell 12/12 system for air-liquid interface (ALI) exposures). For purposes of method development, we used methyl iodide (MeI) as a test compound. The long-term stability of EpiAirway cultures enabled repeated exposures over a 5-d period, which produced responses at lower concentrations than did acute exposure. We are now using these in vitro methods to expose multiple cell culture models (eg, MuCell, AirSmart, AirEp, AlveoLuor) to 1,3-dichloropropene vapor, in order to determine tissue-specific local points of departure (PoDs). Airway dosimetry modeling will be used to in vivo equivalent external concentrations for these PoDs, which can be directly compared to empirically determined values from in vivo studies. The long-term stability of EpiAirway cultures enabled repeated exposures over a 5-d period, which produced responses at lower concentrations than did acute exposure. The long-term stability of EpiAirway cultures enabled repeated exposures over a 5-d period, which produced responses at lower concentrations than did acute exposure. We are now using these in vitro methods to expose multiple cell culture models (eg, MuCell, AirSmart, AirEp, AlveoLuor) to 1,3-dichloropropene vapor, in order to determine tissue-specific local points of departure (PoDs). Airway dosimetry modeling will be used to in vivo equivalent external concentrations for these PoDs, which can be directly compared to empirically determined values from in vivo studies.

### 2847 Is TGF-β1/SMAD3 Signaling Differentially Modulated in Obesity?

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Obesity is associated with severe asthma and poor response to conventional asthma treatments. Human airway smooth muscle (HASM) cells from obese human donors exhibited increased agonist-induced force generation with elevated cytosolic Ca²⁺ flux and myosin light chain (MLC) phosphorylation. Transforming growth factor beta (TGF-β) is a pleiotropic cytokine with pivotal roles in asthma, airway hyperresponsiveness (AHR) and obesity-associated metabolic diseases. Whether TGF-β1 signaling is differentially modulated in HASM in obesity is unknown. To investigate, we hypothesized that TGF-β1/SMAD3 signaling is differentially modulated in obese donors in HASM cells. HASM cells from lean and obese donors pre-treated with vehicle or TGF-β1 (10ng/mL 18 h) were treated with contractile agonist carbachol (CCh 10uM) and lysates were collected. MLC, SMAD3 and protein kinase B (Akt) phosphorylation were determined by immunoblotting. In parallel, interleukin-8 (IL-8) levels were determined in supernatants. Collagen (COL1A1) expression in HASM cells was determined by qRT-PCR. TGF-β1-induced SMAD3 phosphorylation was not significantly different between obese and lean donor derived HASM cells, though obese donors trended towards lower levels compared to lean (Mean±SEM: lean 11.54±8.86 obese 6.45±3.57 n=6 donors/group; p=0.606). Similarly, TGF-β1-induced MLC phosphorylation was comparable between obese and lean donors (lean 0.11±0.04 obese 0.07±0.019 n=4 donors/group; p=0.438). TGF-β1-induced IL-8 release and Akt phosphorylation were comparable between obese and lean donors (IL-8:lean 1199.7±251.3 pg/mg obese 1530.9±740.5 pg/mg n=2 donors/group; pAkt: lean 0.107±0.019 obese 0.078±0.014 n=5 donors/group). Preliminary findings showed that baseline COL1A1 expression was higher in obese donor HASM cells compared to lean by two-fold (fold-change 2.2±0.17 n=1 donor/group), though TGF-β1-induced levels were lower in the obese donors compared to lean (Mean±SEM: lean 1.15±0.41 obese 0.88±0.23 n=1 donor/group). TGF-β1-induced SMAD3 and MLC phosphorylation showed decreasing trends in obese donor HASM cells compared to lean donors, although the differences were not statistically significant. This suggests that TGF-β1/SMAD3 signaling has little effect on defining the obesity phenotype in HASM cells.
Phosgene Oxime (dichloroform oxide; CX), an urticated categorized as a vesicating agent, is a potential chemical threat agent. Its exposure causes rapid and painful dermal injury and systemic effects; however, its mechanism of action is not studied. To elucidate the mechanism of CX-induced toxicity from its dermal exposure, we exposed the dorsal skin of SKH-1 hairless mice to CX 0.5 or 1.0 min using two 12 mm vapor caps. Clinical data from our study showed that CX exposure leads to acute skin lesions (edema, erythema, necrosis, urticaria and blanching) as well as decreases in heart and respiratory rate and drop in body temperature indicating urticaria and anaphylaxis. Skin histopathological analysis showed a CX-induced increase in the epidermal, and dermal plus hypodermal thickness, and apoptotic cell death. Hyperkeratosis, scab formation and macrophage infiltration were observed only in the skin of male mice at 14 day post-0.5 min CX exposure. The skin urticaria following CX cutaneous exposure appears similar to human allergic urticaria, which involves mast cell degranulation that can trigger the release of inflammatory mediators, cytokines, histamine, tryptase and chymase, and reactive oxygen species (ROS). CX exposure may act as secondary messengers in the induction of several biological responses, and damage DNA proteins and lipid. CX exposure in mice induced mast cell degranulation within 30 min of its exposure at both exposure durations and was associated with increased histamine and tryptase levels. CX exposure also caused increased expression of DNA damage (H2AX & p53) and inflammatory (COX2, matrix metallopeptidase 9 & myeloperoxidase) markers within 30 min of its exposure. In male mice, these markers were upregulated till 14 days post-exposure. CX exposure caused increased in a number of pro-inflammatory cytokines and chemokines including IL1α, IL6, vascular endothelial growth factor, and CXCL1 in the skin tissue. CX exposure also decreased the expression of anti-inflammatory cytokines like IL4 and IL10. Lipid peroxidation and oxidative DNA damage were noted as increases in 4-hydroxynonenal adduction formation and 8-oxo-deoxyguanosine expression, respectively, in both 0.5 and 1 min CX exposed skin tissue. CX exposure also resulted in higher phosphorylation levels of cFos and cJun subunits of Activator Protein 1 complex in the skin tissue. Further molecular analysis is underway to determine if the mast cell activation and related signaling pathways could be important contributors in the CX-induced dermal inflammation and toxicity.

Human skin receives exposure to electronic cigarette (EC) refill fluids and the exhaled EC chemicals deposited on indoor surfaces. However, the effects of EC products on skin are poorly understood. In this study, we characterized the effects of EC refill fluids and EC exhaled aerosol residue (ECEAR) on cultured keratinocytes and a 3D human skin model (MatTek EpiDerm®). Flavor chemical and nicotine quantification of Dewberry Cream and Churrios refill fluids was done by GC-MS. Major flavor chemicals (>1mg/mL) in Dewberry Cream were maltol, ethyl maltol, vanillin, ethyl vanillin, and furaneol, while Churrios contained ethyl maltol, benzyl alcohol, vanillin, and ethyl vanillin. Churrios was cytotoxic to human keratinocytes in the MTT assay, which measured mitochondrial reductase activity. Both fluids induced reactive oxygen species (ROS) production in the medium (Ros-Glo™ H2O2 assay) and in cells (CellROX®). 3D MatTek EpiDerm™ skin tissues were exposed at the air-liquid interface to various concentrations of Dewberry Cream, Churrios, and lab made refill fluids for either 4 or 24 hours. Fluids were not cytotoxic in the MTT or LDH assay and did not alter the histology of EpiDerm; however, both fluids induced secretion of inflammatory markers (IL-1α, IL-6, and MMP-9). Three exposure protocols were used with propylene glycol (PG)/ethyl maltol. None produced an effect in the MTT or LDH assay, however all increased secretion of IL-1α and MMP-9. When EpiDerm was exposed to lab made refill fluids (PG and flavor chemicals), secretion of IL-1α was induced, most likely by the PG; however, cytotoxicity was not affected. ECEAR was produced by a participant who used both Dewberry Cream and Churrios over 5 days in a lab-controlled setting. EpiDerm™ was exposed to ECEAR and ECEAR extract. ECEAR did not induce cytotoxicity in the MTT or LDH assay; however, Churrios ECEAR extract induced secretion of IL-1α. In conclusion, refill fluids increased ROS, inflammatory cytokines in keratinocytes, and EpiDerm™ IL-1α secretion was attributed to PG, not the tested flavor chemicals. ECEAR induce cytotoxicity in the MTT or LDH assay; however, Churrios ECEAR extract did not activate an inflammatory response in the EpiDerm. These data are consistent with the conclusion that chemicals in EC refill fluids and ECEAR can cause an inflammatory response in the skin.

2849 Characterizing the Properties of Respirable Silica Particles to Determine Their Role in the Development of Silicosis
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Silicosis is an irreversible, fibrotic respiratory disease associated with occupational exposures to silica particles in numerous sectors, including mining, construction, and manufacturing. Traditionally, the toxicity of silica exposures has been assessed by the mass concentration of particles deposited in the lungs. However, other factors, such as size distribution, surface properties, shape, or form (e.g., crystalline or amorphous), have been demonstrated to also contribute to toxic responses. A systematic literature review based on the approaches established by the EPA, NTP, and other organizations was conducted to identify and integrate relevant scientific information on the properties of silica particles that can affect lung toxicity. The following properties of silica were included in the analysis: concentration, size, shape, surface properties (e.g., pieoelectric effects), form (i.e., crystalline vs. amorphous), and surface area. Additionally, a data gap analysis was conducted to elucidate the degree of evidence for toxicological impacts of each property. The results of the analysis revealed that the toxicity of silica particles depends on a combination of factors. Particle size, for example, is a known key factor in silica toxicity; however, the relationship between toxicity and size of silica particles is complex. At the cellular level, particle size impacts both reactive surface area and the probability of uptake by alveolar macrophages. These characteristics can work in opposite directions depending on the specific particle size and total concentrations. Similar dose-dependent complexities exist for the other properties of silica particles as well. Overall, the analysis revealed that many of these physicochemical properties contribute to the onset of silicosis and the reduction of the overall exposure (i.e., the cumulative dose) remains the most feasible method to reduce the risk of adverse effects. Addressing the identified data gaps will refine scenario-based risk assessments with advanced dose metrics.

2850 E-cigarette Fluids and Exhaled Residue Cause an Inflammatory Response in Both Human Keratinocytes and a 3D Skin Model
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Physical skin receives exposure to electronic cigarette (EC) refill fluids and the exhaled EC chemicals deposited on indoor surfaces. However, the effects of EC products on skin are poorly understood. In this study, we characterized the effects of EC refill fluids and EC exhaled aerosol residue (ECEAR) on cultured keratinocytes and a 3D human skin model (MatTek EpiDerm®). Flavor chemical and nicotine quantification of Dewberry Cream and Churrios refill fluids was done by GC-MS. Major flavor chemicals (>1mg/mL) in Dewberry Cream were maltol, ethyl maltol, vanillin, ethyl vanillin, and furaneol, while Churrios contained ethyl maltol, benzyl alcohol, vanillin, and ethyl vanillin. Churrios was cytotoxic to human keratinocytes in the MTT assay, which measured mitochondrial reductase activity. Both fluids induced reactive oxygen species (ROS) production in the medium (Ros-Glo™ H2O2 assay) and in cells (CellROX®). 3D MatTek EpiDerm™ skin tissues were exposed at the air-liquid interface to various concentrations of Dewberry Cream, Churrios, and lab made refill fluids for either 4 or 24 hours. Fluids were not cytotoxic in the MTT or LDH assay and did not alter the histology of EpiDerm; however, both fluids induced secretion of inflammatory markers (IL-1α, IL-6, and MMP-9). Three exposure protocols were used with propylene glycol (PG)/ethyl maltol. None produced an effect in the MTT or LDH assay, however all increased secretion of IL-1α and MMP-9. When EpiDerm was exposed to lab made refill fluids (PG and flavor chemicals), secretion of IL-1α was induced, most likely by the PG; however, cytotoxicity was not affected. ECEAR was produced by a participant who used both Dewberry Cream and Churrios over 5 days in a lab-controlled setting. EpiDerm™ was exposed to ECEAR and ECEAR extract. ECEAR did not induce cytotoxicity in the MTT or LDH assay; however, Churrios ECEAR extract induced secretion of IL-1α. In conclusion, refill fluids increased ROS, inflammatory cytokines in keratinocytes, and EpiDerm™ IL-1α secretion was attributed to PG, not the tested flavor chemicals. ECEAR also activated an inflammatory response in the EpiDerm. These data are consistent with the conclusion that chemicals in EC refill fluids and ECEAR can cause an inflammatory response in the skin.

2851 Mechanism Mediating the Dermal Inflammation and Toxicity from Phosgene Oxime Exposure
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Emerging evidence suggests that environmental chemicals including major ubiquitous organic pollutants like polycyclic aromatic hydrocarbons (PAHs) could contribute to the pathophysiology and high prevalence of chronic inflammatory skin diseases like atopic dermatitis and psoriasis. Benzo[a]pyrene (BaP), the main source of atmospheric PAH, is generated mainly from cigarette smoke, wood-burning and automobile exhaust. To investigate the effect of BaP exposure on skin inflammation, we generated a mouse psoriatic model: the dorsal skin of naive C57BL/6 mice was shaved and exposed to 62.5mg of 5% imiquimod (IMQ) cream once daily for five days. For assessing the initiation or exacerbation of psoriasis from exposure to BaP, mice were exposed 64µg BaP in 50µl acetone for five days before IMQ application (BaP+IMQ) or for five days together with the IMQ (BaP+IMQ) application. Following the analysis of clinical lesions, mice were sacrificed, and skin sections were analyzed for inflammation-related histopathological and molecular changes. Our results showed that BaP exposure together with IMQ exacerbated IMQ-induced psoriatic inflammatory symptoms including the skin bi-fold thickness, epidermal and dermal thickness, hyperkeratosis, dermal fibrosis, neutrophil infiltration, neutrophil degranulation, and mast cell degranulation. Molecular mechanisms surrounding the initiation and exacerbation of psoriasis following exposure to environmental contaminants like BaP are understudied, though there are strong indications that these responses are mediated by aryl hydrocarbon receptor (AhR) activation. AhR can bind to PAH’s with high affinity and induce oxidative stress, and AhR ligands can exert antioxidative activity by activating antioxidant transcription factor nuclear factor-erythroid 2-related factor-2 (NRF2). Our ongoing molecular studies revealed that exposure of BaP...
in IMQ-induced psoriatic mouse model elevated the protein expression of inflammatory markers like COX-2 and MMP9. We are currently analyzing the oxidative stress and inflammatory markers, inflammatory cytopathies, and the role of AhR and NRF2 as well as associated signaling pathways in the pathogenesis of psoriasis following BaP exposure.

2953 Liquid Smoke-Induced Abnormal Cornified Envelope Formation in Human Keratinocyte


Skin functions as a physical and permeability barrier that prevents the loss of water and the entry of environmental toxins and infectious microbes. The protection provided by the skin, however, is not unlimited. Increasing numbers of studies indicate that prolonged or repetitive exposure to high levels of air pollutants may cause profound negative effects on the skin, including aging, inflammatory responses, and cancer. Smoke from forest fires and residential wood burning are major contributors to both indoor and outdoor harmful air quality. In this study, we report that liquid smoke (LS), generated by aqueous extraction of condensed wood smoke, can react with and cross-link keratinocyte intracellular proteins, leading to abnormal cornified envelope (CE) formation. The expression of genes ordinarily involved in keratinocyte differentiation (which culminates normally in CE formation) were hardly altered by LS exposure. Instead, the expression of genes associated with oxidative stress, pro-inflammatory response, and xenobiotic metabolism were upregulated by LS. Transglutaminase (TGM1) is a crucial enzyme for catalyzing CE formation as it mediates protein cross-linking under the plasma membrane of a differentiating keratinocyte. When the activity of TGM was abrogated with iodoacetamide, LS-stimulated CE formation and protein cross-linking in keratinocytes instead of normal keratinocytes, the formation of LS-induced CEs could not result from oxidative stress and protein adducts from aldehydes (known components of wood smoke), as pre-treatment of LS with sodium borohydride (which reduces aldehydes to alcohols) decreases the ability of LS to promote protein cross-linking and CE formation. Finally, LS-induced CEs were shown to have higher protein content than normal CEs, suggesting that aldehydes could introduce abnormal protein incorporation into CEs, and thereby impair their function as a barrier. This work reveals an unanticipated adverse impact of pro-inflammatory air pollutants on skin health.

2954 Validation of Restricted Substances Lists and Chemical Hazard Classifications as Screening Tools for Identifying Potential Skin Sensitizers in Consumer Products

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Restricted substances lists (RLSs) are typically created to provide guidelines for consumer product companies to comply with relevant human health and environmental regulations, standards, and to address general product safety concerns. RLSs, along with chemical hazard classifications, can therefore be considered tools for controlling or reducing the use of hazardous chemicals in consumer products. Focusing on hazard classification lists for skin sensitization and RLSs for allergic substances in skin-contacting consumer products, we investigated correlations between a chemical's inclusion on one or more lists with the conclusions of hazard assessments based on experimental data. From a pool of 126 lists identified from the List of Lists (LOL) database using the search terms “sensitizer,” “sensitization,” “allergic,” or “allergen,” we excluded lists that were duplicated or irrelevant to skin sensitization (e.g., respiratory sensitization), bringing the total number to 22. The remaining lists were then categorized into two tiers: Tier I lists were those developed by recognized experts and/or authoritative regulatory bodies (e.g. European Union’s harmonized hazard classifications [CLP]) while Tier II lists were those developed using a less comprehensive review, estimated data, or compiled by an organization not considered to be authoritative (e.g. non-harmonized GHS classifications). For this work we conducted a proof of concept study to investigate the applicability domain of GARDskin and GARDpotency, addresses Key Event 3 (dendritic cell activation) of the skin sensitization Adverse Outcome Pathway, and provides reliably potency information for several chemical classes. Understanding of the applicability domain of test methods is pivotal in providing confidence in assay outcomes, facilitating regulatory uptake in specific industry sectors. The purpose of this work is to verify the applicability domain of GARDskin and GARDpotency, for the product class of agrochemical formulations. For this proof of concept, 20 agrochemical formulations were tested using GARDskin. When GARDskin was positive, GARDpotency assay was used to determine the severity of sensitization potential. Tests were conducted according to the Standard Operating Procedures. The selected agrochemical formulations were liquid (11 water based; and 9 organic solvent based) with a balanced ratio of strongly acidic (3,14), neutral (11), and weakly acidic (3) RSL compositions. The results of this proof of concept study will be presented.
Proactive identification and characterization of sensitization hazards are central aspects of risk assessment of chemicals. Current legislation and trends in predictive toxicology advocate a transition from in vivo methods to non-animal alternatives, with a number of methods for hazard assessment of skin sensitizers currently available. However, non-animal methods capable of providing quantitative assessment of sensitizing potency are currently lacking. The GARDskin assay is a next-generation in vitro assay for hazard assessment of skin sensitizers, currently progressing towards regulatory acceptance. Recently, the GARDskin Dose-Response (DR) testing strategy was introduced, in which test chemicals are evaluated by the GARDskin assay in a titrated range of concentrations, in order to investigate the dose-response relationship between GARDskin skin classifications and test concentration. As such, it provides a quantitative estimation of sensitizing potency, referred to as cDV0, which corresponds to the least required dose able to generate a positive response in the GARDskin assay. The cDV0 value obtained for a test chemical may be viewed as an analogue to the LLNA EC3 value, based on which further hazard characterization and risk assessment may be performed. Statistically significant correlation between the GARDskin DR cDV0 and the LLNA EC3, as well as with human No Expected Sensitization Induction level (NESSIL) estimations has been confirmed, thus enabling direct extrapolation between the different metrics. Here, we further introduce the GARDskin DR protocols, as proposed in a standardized testing strategy. By studying a concentration range of 6 concentration points titrated from the experimentally derived GARD skin input concentration in biological duplicates, a test chemical-specific cDV0 is established by linear interpolation. We illustrate how these results can be used on their own to facilitate direct potency-associated subcategorization of test chemicals according to UN GHS classification criteria. Lastly, we illustrate how results generated with GARDskin DR can be directly incorporated into existing strategies for Quantitative Risk Assessment using an entirely in vitro setup.

Adequate levels of thyroid hormone (TH) are needed for proper fetal and early life stage brain development. Exposure to thyroid disrupting chemicals (TDCs) can lead to deficiencies of serum THs during pregnancy, depriving the fetal brain of hormone and compromising neurodevelopment. Regulatory assessment of TDCs is largely informed by chemical disruption of serum hormone concentrations in rodent models. High throughput (HTP) in vitro assays of several biochemical pathways of thyroid hormone synthesis and metabolism are used to screen chemicals for their potential to disrupt the thyroid axis. Computational toxicology strives to translate in vitro based screening information to predict organisinal outcomes of potential TDCs. To this end, estimates of target tissue concentrations are required (i.e. chemical and hormone concentrations in the serum, thyroid gland, and liver) during pregnancy and early-life stages. Chemical tissue concentrations are controlled by pharmacokinetic determinates such as absorption, distribution, metabolism and excretion (ADME). Thyroid hormone concentrations in serum are also influenced by TH kinetics of synthesis, distribution, catabolism, metabolism, and transport via carriers. We report here an integrative computational model including life-stage physiologically based pharmacokinetic (PBPK) and TH kinetic models. This model implements a mechanistic quantitative approach to translate TH disruption in vitro HTP assays to in vivo exposures of circulating THs serum level in a pregnant mother, the fetus and the neonate. It was developed and calibrated using literature data on basal and disrupted levels of the hormones during pregnancy in the mother, fetus, newborn and neonate. When combined with quantitative methods and literature data for PBPK chemical specific parameters, this integrative quantitative approach can be generalized across many chemicals and exposure scenarios to augment regulatory decision making. This abstract does not necessarily reflect US EPA policy.

Adequate levels of thyroid hormone (TH) are needed for proper fetal and early life stage brain development. Exposure to thyroid disrupting chemicals (TDCs) can lead to deficiencies of serum THs during pregnancy, depriving the fetal brain of hormone and compromising neurodevelopment. Regulatory assessment of TDCs is largely informed by chemical disruption of serum hormone concentrations in rodent models. High throughput (HTP) in vitro assays of several biochemical pathways of thyroid hormone synthesis and metabolism are used to screen chemicals for their potential to disrupt the thyroid axis. Computational toxicology strives to translate in vitro based screening information to predict organisinal outcomes of potential TDCs. To this end, estimates of target tissue concentrations are required (i.e. chemical and hormone concentrations in the serum, thyroid gland, and liver) during pregnancy and early-life stages. Chemical tissue concentrations are controlled by pharmacokinetic determinates such as absorption, distribution, metabolism and excretion (ADME). Thyroid hormone concentrations in serum are also influenced by TH kinetics of synthesis, distribution, catabolism, metabolism, and transport via carriers. We report here an integrative computational model including life-stage physiologically based pharmacokinetic (PBPK) and TH kinetic models. This model implements a mechanistic quantitative approach to translate TH disruption in vitro HTP assays to in vivo exposures of circulating THs serum level in a pregnant mother, the fetus and the neonate. It was developed and calibrated using literature data on basal and disrupted levels of the hormones during pregnancy in the mother, fetus, newborn and neonate. When combined with quantitative methods and literature data for PBPK chemical specific parameters, this integrative quantitative approach can be generalized across many chemicals and exposure scenarios to augment regulatory decision making. This abstract does not necessarily reflect US EPA policy.
ized framework. To that end, we have developed the Population Life-course Exposure to Health Effects Model (PLETHEM), a modular open source modeling platform that provides users the ability to create, run, share, and audit PBPK models. The platform consists of a database of chemicals, QSAR models, life-stage specific physiological and metabolic parameters needed to parameterize PBPK models, an R-based engine to perform model simulations, and an interactive user interface to define and select parameter sets for the models. PLETHEM implements easy to use interfaces for a generic PBPK model and a high-throughput IVIVE model. The PLETHEM database also incorporates ontogeny profiles for key metabolic enzymes that can be used to calculate in vivo metabolic clearance using measured in vitro clearance. In addition, PLETHEM enables an ability to link to several EPA and OECD exposure estimation programs. These models, which estimate exposures in the workplace and the general populations, can be used to drive PBPK model-based estimates of resulting internal exposures to support risk assessments. Recently we have updated PLETHEM to include PBPK modeling trout. We have also released a ready to use online version of PLETHEM (https://www.scitovation.com/plet- hemisphere) in addition to the R package available from CRAN (The Comprehensive R Network Archive). We have created several workflows to guide the novice user through all of PLETHEM’s functionality, available in the same location as the online version.

2962 Mass Balance Model for Simulation of In Vitro Dynamic Chemical Distribution with Repeat Dosing

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As toxicologists move away from animal testing and more toward the use of in vitro models and biological modeling, it is necessary to produce tools to illuminate the distribution of the chemical within the in vitro environment. Although models predicting chemical distribution in vitro have been developed, very little has been done for repeated dosing scenarios, which are common in protracted experiments where medium needs to be refreshed. Failure to account for repeated dosing may lead to inaccurate estimations of cellular concentrations and introduce bias into subsequent in vitro to in vivo extrapolations. For this study, our aim is to develop a dynamic mass balance model for repeated dosing in in vitro systems and to assess the impact of repeated doses on cellular concentrations. To accomplish our objective, we used a previous single-dose partitioning-based mass balance model and modified it to account for time-related changes in the in vitro environment. Using this modified model, we simulated repeated dosing scenarios for hypothetical organic chemicals with a range of partitioning properties and compared the in vitro distributions (i.e., predicted concentrations in medium, cells, and plastic) over time. Results produced by the model showed that the maximum cellular concentration greatly differed based on the dosing regime and medium characteristics. For example, for an in vitro test over 7 days with a chemical with a logKow of 7 in a medium with a serum albumin volume fraction of 2%, the maximum cellular concentration was 1.8 times higher for repeated dosing (50% of medium changed every other day) compared to a single dose. The maximum cellular concentration was 1.9 times higher with no serum albumin added and 6.4 times lower with a serum albumin volume fraction of 20% under the repeated dosing regimen. In conclusion, the model exhibits the importance of accounting for chemical characteristics, in vitro conditions and dosing regimen when assessing chemical distribution and intracellular concentrations.

2963 Development of High-Throughput In Vitro Human Alveolar Tissue Models Utilizing Novel Electrospun Scaffolds

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Alveolar tissue damage is a hallmark of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and coronavirus disease 2019 (COVID-19). In vitro models of human alveolar air-blood barrier (ABB) tissue are needed for COVID-19 research and therapeutics development. However, high throughput (HTP) formats of these tissue models are lacking. We are developing HTP human ABB tissue models by adapting novel electrospun scaffolds to HTS Transwell®-24 and 96 well permeable support plates (Corning Life Sciences). BioSpin™ biodegradable poly(lactic-co-glycolic acid) (PLGA) scaffolds were prepared using solution electrospinning and post-treatment processes which produced scaffolds with thicknesses as low as 6 μm. Human pulmonary microvascular endothelial cells (HPMEC) were seeded onto the underside (blood-side) of the electrospun scaffold, and normal human alveolar epithelial cells (HAEpiC) were seeded onto the top (air-side). Scaffolds were cultured at the air-liquid interface to produce ABB tissue models. The thin electrospun scaffolds are biodegradable and diminished with time to allow for a more physiologic interaction between the endothelial and epithelial cells as compared to previously described in vitro ABB models. Transepithelial electrical resistance (TER) measurements, utilized to determine the barrier properties of the ABB models, showed stable, long-term TER values as high as 2,300 Ω cm², demonstrating extremely robust epithelial barrier. Immunohistochemistry was used to assess expression of SARS-CoV-2 receptors and cell specific markers. Alveolar epithelial cells are reported to have relatively low expression of angiotensin-converting enzyme 2 (ACE2), a key SARS-CoV-2 receptor, while endothelial cells reportedly express higher levels. Other putative receptors and co-factors for SARS-CoV-2 infection have also been reported. HIC revealed high expression of ACE2 on HPMEC and comparatively low expression by the HAEpiC as expected. These results indicate that HTP in vitro human ABB models produced on novel electrospun scaffolds are unique and useful models for SARS-CoV-2/COVID-19 research and will likely find utility for wider applications in respiratory infection, toxicology and drug delivery as well.

2964 Characterization of Single Species Biofilms in an In Vitro Intestinal Model


The use of in vitro and computational methods in the drug development/safety field has recently shown an exponential increase. The in vitro model of the intestinal epithelium using Caco-2/HT29-MTX has been widely used for pharmacology and toxicology purposes. However, the human gut microbiota, composed mainly of bacteria, is currently in the spotlight due to its critical role in host health. Several studies have emphasized that gut microbiota directly affects host immunity, digestion, gut endocrine function, toxin elimination, and drug action and metabolism. The presence of bacteria in the gut can be either as planktonic (non-adherent cells) or as biofilm communities (bacteria in the mucus layer lining mucosal surfaces and surrounded by extracellular polymeric substances). To achieve a more realistic small intestinal ecosystem in vitro, we aimed to create a gut model incorporating Caco-2/HT29-MTX and a stable, human-derived microbial biofilm. We used common bacterial species found in the duodenum, which included the Gram-positive commensal Lactobacillus rhamnosus GG and Bifidobacterium bifidum VPI 1124, and Gram-positive opportunistic Streptococcus salivarius SS2 and Enterococcus faecalis NCTC 775. Briefly, bacteria were inoculated and allowed to form biofilms in 24-well plates. After 4 days, biofilms were transferred to Caco-2/HT29-MTX co-cultures up to 72 h. Results show that L. rhamnosus and B. bifidum biofilms did not disrupt the intestinal model epithelium stability, while S. salivarius and E. faecalis significantly compromised the epithelium at 12- and 24-hours, respectively. Moreover, viable counts of the supernatant of the supernatant and mucosal epithelium were performed to monitor the adhesive and proliferating ability of the biofilms in the model, which increased throughout the exposure time up to 10²-10³ CFU/mL. Confocal microscopy demonstrated the biofilms colonization and confirmed S. salivarius as the most aggressive effects. Using a bottom-up approach (from components to communities), we have developed a reproducible model of the upper small intestine useful to explore potential causality and related microbiota-mediated disease mechanisms as well as to understand their complex metabolic interactions.

2965 Utilizing Computational Fluid Dynamics Modeling to Create an Aerosol-Specific Cell Culture Exposure System to Evaluate the Toxicity of Aerosols at the Air-Liquid Interface


Traditional in vitro studies utilize submerged exposures in which toxicants are solubilized in water or dimethyl sulfoxide (DMSO) and added to cell culture media. However, approximately 30% of compounds nominated for study in the U.S. Environmental Protection Agency’s (EPA) Toxic Substances Control Act (TSCA) inventory are insoluble or volatile, and therefore cannot be adequately tested using traditional in vitro dosing methods. To address these challenges, the cell culture exposure system (CCES) was developed to expose human bronchial epithelial cells maintained at air-liquid interface (ALI) to volatile organic compounds (VOCs). The CCES successfully delivers six concentrations of VOCs to four technical replicates within a 24-well format which
allows medium-throughput testing of volatile compounds. However, insoluble and nonvolatile compounds must be generated and delivered as aerosols. We optimized an aerosol generation system that delivers a monodispersized aerosol population (d=1.4-1.6 μm) to the dilution manifold of the CCES. To quantify CCES performance in delivering aerosols, a fluorescent tracer was delivered through the system and its deposition was quantified. Results revealed that the VOC and CCEs in vitro and in vivo recapitulate many of the functional cellular and molecular characteristics of differentiated human primary proximal tubule cells, including anchorage dependence, gluconeogenesis capability, and sodium-dependent sugar transport. To evaluate its ability to deliver data that is concordant with published outcomes, we selected cell pure from animal TDCs studies are sometimes unreliable due to species differences in TDCs’ modes of action (MOA). However, immortalized human thyroid cells typically lose key thyroid specific functions and/or follicular epithelial architecture when cultured in 2D monolayers. No available primary or normal thyroid cell lines exhibit measurable levels of TH synthesis to screen TDCs. Currently, there is an unmet need for a reliable in vitro human thyrocyte model to investigate TDC-induced thyroid toxicity. In this study, our goal was to develop a bank of cryopreserved primary human thyrocytes qualified for thyroglobulin (TG) and thyroxine (T4) production as reference cigarette for this study. The smoke-shunt exposure system (ECCM) to evaluate the inflammatory effects induced by smoke. The 3R4F was tested as reference cigarette for this study. The smoke-shunt exposure system was used for whole cigarette smoke exposure. The Beas-2b cells were exposed to 5 different smoke doses (0.125*2,4,6,8 puff) by shunt system. CCK-8 was used to detect the cell viability to determine the smoke exposure dose in the lung unit (Beas-2b) cells in the ECCM. The doses of 0.125*3 puff air and 0.125*3 puff smoke were selected to expose the lung unit (Beas-2b) cells in ECCM with metabolic function (human 25 donor liver microsomes) and non-metabolic function (human 25 donor liver microsomes). After the exposure, the fluid in the model continued to circulate for 3 hours, then the liver microsomes were taken out and the cells in the three units were co-cultured for 24 hours for detection. CCK-8 and ELISA were used to detect the cell viability and IL-8 secretion levels in the lung unit (Beas-2b), vascular unit (HUVEC) and circulating unit (human lung macrophages) in the two models, respectively. The study found that with the increasing number of puff (0.125*2,4,6,8), the survival rate (100%, 90%, 85%, 77%, 43%) of Beas-2b cells in the exposure chamber continued to decline. Compared with the model with no metabolic function (cell viability (%): Beas-2b, 37; HUVEC, 96; human lung macrophages, 101. IL-8 (pg/mL): Beas-2b, 304; HUVEC, 1076; human lung macrophages, 8), the cell viability in the lung unit and the circulation unit in the metabolic function model (cell viability (%): Beas-2b, 81; HUVEC, 99; human lung macrophages, 138. IL-8 (pg/mL): Beas-2b, 844; HUVEC, 1188; human lung macrophages, 13) increased significantly, and the secretion levels of IL-8 in the lung unit increased significantly. These results indicate that the ECCM with metabolic function can significantly reduce the damage to cells and promote inflammation in the lung unit caused by smoke. This research was supported by grants from Dean’s Science and Technology Development Fund Project (322017CA0140) of Zhengzhou Tobacco Research Institute of CNTC.

**2967 Using the Human Proximal Tubule Cell Line HK-2 to Evaluate In Vitro to In Vivo Concordance of Toxicity**

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Prominent use of the renal proximal tubule cell line, Human Kidney 2 (HK-2), as an in vitro cellular model in toxicology studies prompted us to investigate how well these cells can reliably reveal the toxicological profile of compounds having a potential to cause proximal tubule injury in vivo. HK-2 cells effectively recapitulate many of the functional cellular and molecular characteristics of differentiated human primary proximal tubule cells, including anchorage dependence, gluconeogenesis capability, and sodium-dependent sugar transport. To evaluate its ability to provide data that is concordant with published outcomes, we selected cell pure from animal TDCs studies are sometimes unreliable due to species differences in TDCs’ modes of action (MOA). However, immortalized human thyroid cells typically lose key thyroid specific functions and/or follicular epithelial architecture when cultured in 2D monolayers. No available primary or normal thyroid cell lines exhibit measurable levels of TH synthesis to screen TDCs. Currently, there is an unmet need for a reliable in vitro human thyrocyte model to investigate TDC-induced thyroid toxicity. In this study, our goal was to develop a bank of cryopreserved primary human thyrocytes qualified for thyroglobulin (TG) and thyroxine (T4) production as reference cigarette for this study. The smoke-shunt exposure system (ECCM) to evaluate the inflammatory effects induced by smoke. The 3R4F was tested as reference cigarette for this study. The smoke-shunt exposure system was used for whole cigarette smoke exposure. The Beas-2b cells were exposed to 5 different smoke doses (0.125*2,4,6,8 puff) by shunt system. CCK-8 was used to detect the cell viability to determine the smoke exposure dose in the lung unit (Beas-2b) cells in the ECCM. The doses of 0.125*3 puff air and 0.125*3 puff smoke were selected to expose the lung unit (Beas-2b) cells in ECCM with metabolic function (human 25 donor liver microsomes) and non-metabolic function (human 25 donor liver microsomes). After the exposure, the fluid in the model continued to circulate for 3 hours, then the liver microsomes were taken out and the cells in the three units were co-cultured for 24 hours for detection. CCK-8 and ELISA were used to detect the cell viability and IL-8 secretion levels in the lung unit (Beas-2b), vascular unit (HUVEC) and circulating unit (human lung macrophages) in the two models, respectively. The study found that with the increasing number of puff (0.125*2,4,6,8), the survival rate (100%, 90%, 85%, 77%, 43%) of Beas-2b cells in the exposure chamber continued to decline. Compared with the model with no metabolic function (cell viability (%): Beas-2b, 37; HUVEC, 96; human lung macrophages, 101. IL-8 (pg/mL): Beas-2b, 304; HUVEC, 1076; human lung macrophages, 8), the cell viability in the lung unit and the circulation unit in the metabolic function model (cell viability (%): Beas-2b, 81; HUVEC, 99; human lung macrophages, 138. IL-8 (pg/mL): Beas-2b, 844; HUVEC, 1188; human lung macrophages, 13) increased significantly, and the secretion levels of IL-8 in the lung unit increased significantly. These results indicate that the ECCM with metabolic function can significantly reduce the damage to cells and promote inflammation in the lung unit caused by smoke. This research was supported by grants from Dean’s Science and Technology Development Fund Project (322017CA0140) of Zhengzhou Tobacco Research Institute of CNTC.


The devTOX quickPredict platform developed by Steminia Biomarker Discovery is a human pluripotent stem cell-based assay that predicts the potential of a molecule to result in developmental toxicity based on metabolic perturbations following chemical exposure. Using eight concentration responses per compound, Syngenta have profiled over fifty research molecules through the assay to determine the perturbation in the ratio of ornithine to citrulline in the stem cell media and derive points of departure (POD) for developmental toxicity prediction. The predictions were tested for a subset of 31 molecules using data from preliminary developmental toxicity studies, traditionally used to inform on developmental toxicity risk in research projects. Assay performance reached 63% accuracy with low specificity (35%) but high sensitivity (>90%) when compared to the in vivo data. However, by comparing the in vitro POD with the achieved in vivo steady state systemic blood concentration (Cmax) from repeat dose toxicity studies, the overall accuracy of the assay was drastically improved. Assay performance increased to 87% with high specificity (>81%) and high sensitivity (>92%). As a result of this analysis Syngenta Product Safety have been employing the devTOX qP assay on research projects to enable decision making at much earlier stages in new active ingredient development. Future work will address whether exposure corrections using single dose kinetic data, or even modelled exposure data, is adequate for acceptable levels of predictivity.

**2968 The Human In Vitro Developmental Toxicity Assay, devTOX quickPredict, Accurately Predicts the Developmental Toxicity Potential of Agrochemical Research Molecules**

Assessing the accuracy, reproducibility, and applicability domain of new approach methods (NAMs) is necessary step for establishing confidence in these methods and enabling their use in a regulatory setting. Over 100 chemicals have been evaluated with the devTOX quickPredict (devTOX®) assay, which predicts the developmental toxicity potential of a chemical based on changes in human iPS cell metabolism. The assay predicted the developmental toxicity potential across this diverse set of chemicals with 87% accuracy (88% sensitivity, 86% specificity). Within individual chemical use classes (i.e., pharmaceuticals or pesticides), assay accuracy ranged from 81% to 94%, demonstrating the broad applicability of the assay. To further define the assay’s applicability domain, the results were separated into different pharmacological categories and performance was assessed. The assay’s sensitivity in these pharmacological categories ranged from 50% to 100% and provides insight into the assay’s biological applicability domain. For example, the assay had 100% sensitivity for developmental toxicants classified as channel, kinase, and transcription modulators and DNA modifiers. In contrast, receptor modulators were predicted with 50% sensitivity, and were highly dependent upon whether the iPS cells expressed the specific receptor being modulated. The reproducibility of the predictive model was evaluated using independent replicates of three chemical treatments (carbamazepine, n=34; methotrexate, n=34; thalidomide, n=9) conducted by multiple technicians with multiple iPS cell lines, freeze lot and reagents over the course of 5 years. The interpolated developmental toxicity potential (dTP) values (determined using the devTOX® predictive model) were within two standard deviations of the mean for each of the chemicals, demonstrating that the assay endpoints are reproducible over time. These data demonstrate the importance of understanding a NAM’s biological system, its strengths and its limitations. Taken together, these data demonstrate the accuracy, reproducibility and broad applicability domain of the devTOX® assay and support its use as an alternative to animal models for developmental toxicity testing.
2974 Biomarkers of PAH Toxicity in Normal and Asthmatic Human Bronchial Epithelial Cells
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There is increased emphasis on understanding cumulative risk from the com-
bined effects of PAHs and other environmental pollutants as they relate to pub-
lic health. Recent animal studies have identified pulmonary inflammation as a 
possible modifier and risk factor for chemical toxicity in the lung after expo-
sure to inhaled pollutants; however, little is known about specific interactions 
and potential mechanisms of action. In this study, primary human bronchial epithelial cells (HBE) cultured in 3D at the air-liquid interface are utilized as a 
physiologically relevant model to evaluate the effects on toxicity of polycyclic aromatic hydrocarbons (PAHs), a class of contaminants generated from incomplete combustion of fossil fuels. These studies evaluate the effects of benzo(a)pyrene (BaP), which is a commonly studied PAH, in 
HBEC exhibiting a normal and asthmatic phenotype by comparing end-
points of cytotoxicity, barrier integrity and metabolic function. Both normal 
cells from healthy donors and normal cells pre-treated with IL-13 during 
differentiation, which induces an asthmatic phenotype, were treated with 40 µg/ 
ml benzo(a)pyrene (BaP) or 1% DMSO/PBS vehicle control for 48 hrs. Cells 
with the asthmatic phenotype treated with BaP showed increased cytotoxicity, 
decreased barrier integrity and decreased metabolic function, compared to normal cells. In addition, global gene expression as measured by RNA sequencing resulted in a large number of differentially expressed genes between normal and dis-
cessed cells with BaP treatment suggesting that individuals with pre-existing inflammatory lung disease have altered susceptibility to chemical contam-
ants in air pollution. Future studies will focus on mRNAs and miRNAs as 
biomarkers of exposure and disease.

2975 Repeatability of a Depth of Injury Test Method for the Classification of Eye Irritants
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The purpose of this study is to evaluate variability in the newly developed Globally Harmonized System (GHS) prediction model for classifying ocular irritant materials based on depth of injury (DoI) measurements. Previously, 16 different materials were tested, representing all classes of toxicity, according to the GHS classification systems. The new study used a subset of these chemicals to determine the reproducibility of the method. For this method, food source rabbit eyes were used. Tissues were exposed to test material for 1 min, and corneas were collected 24 hours after exposure. Tissues were then fixed and processed for live/dead biomarker fluorescent staining using phalloidin. DoI was then measured, and the percent DoI values for the stroma were compared to the prediction model. The GHS not classified (NC) irritant, n-hexyl bromide (CASRN 111-25-1), caused no damage to the stroma with an average depth of injury of 0% and a standard deviation of 0%. The GHS category 2 material, n-octanol (CASRN 111-87-5), caused damage to the stroma with an average depth of injury of 12.7% with a standard deviation of 5.0%, whereas the GHS corrosive, cyclohexanol (CASRN 108-93-0), caused significantly greater damage to the stroma with an average depth of injury of 44.4% with a standard deviation of 4.3%. The data from ongoing independent replicates for the same chemicals demonstrate that the method is statistically reproducible with low variability.

2976 Integrating Cytotoxicity Profiling into the H295R Steroidogenesis Assay
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The OECD validated H295R steroidogenesis assay (OECD test guideline 456) utilizes human adrenocortical carcinoma cells to evaluate chemical effects on testosterone (T) and 17β-estradiol (E2) production. This assay requires a par-
alleled measure of cytotoxicity, generally by MTT. With steroidogenesis being a 
process whose rate limiting and commitment steps depend on mitochondrial 
hormone production. Cumulatively, this study reveals that comprehensive 
assessment of cytotoxicity, as determined by probing cell and mitochondrial 
status, can complement hormone data to better understand and characterize 
mechanism of action for chemical-mediated disruption of steroidogenesis.

2977 Toward Automation of High-Throughput Toxicology Assay Image Analysis Using Deep Learning
A. Tandon et al. 

Automated approaches to perform high throughput toxicology often employ microscopy to capture photomicrographs from multi-well cell culture plates generating thousands of images that require time-consuming human analysis. To automate this subjective and time-consuming manual process, we have developed a deep learning system that uses deep learning to automatically classify digital assay images. We have trained a convolutional neural network (CNN) to perform binary and multi-class classification. The binary classifier classified assay images into healthy (comparable to vehicle controls) and altered (not comparable to vehicle-control) classes with >98% accuracy; the multi-class classifier classified assay images into healthy, stressed and altered classes with >93% accuracy. Our dataset comprised 2,156 and 2,254 high-resolution assay images from proliferated and differentiated 2D cultures of HepG2 cells, re-
spectively. The dataset was annotated into 4 classes by expert annotators: Cell-Loss, Stress, Normal, and Normal. In this study we have focused on the top 7 scoring unmixing variants (CNN architectures, including ResNet 14, 50 and 101). To visualize regions in the images that the CNN model used for clas-
sification, we employed Class Activation Maps (CAM). This allowed us to better understand the inner workings of the neural network and led to further optimizations of the algorithm. Finally, we are currently exploring various methods of performing deep learning classification on the classification scores, with preliminary results suggesting that these scores might be used as an alternative method of computing benchmark dose. Together, these results clearly demonstrate that deep-learning based image classification of cell toxicity can yield highly accurate results that are automated and reproducible.

2978 Effects of p-cresol and Conjugated Metabolites on Markers of Cellular Toxicity in HepaRG Cells

p-Cresol (PC) is a uremic toxin that can be accumulated to high concentra-
tions (i.e., >0.5 mM including metabolites). PC and its metabolites (i.e. glucu-
ronide [PCG] and sulfate [PCS] conjugates) are associated with cardiovascular outcomes. The objectives of this study were to determine the relative effects of PC (vs. exogenously administered metabolites) and the roles of in situ generated metabolites on the manifestation of cellular toxicity in HepaRG cells. Concentration-
(0-2mM) and time-dependent (0-24hr) effects of exogenously generated 
metabolites on markers of oxidative stress (2’,7’-dichlorofluorescein, DCF, formation), cellular glutathione (GSH) depletion, and necrosis (lactate dehydrogenase, LDH, release) were charac-
terized. The relative effects of PC, PCS, PCG on all toxicity markers were 
compared. Concentrations of PC and metabolites in culture were determined by validated assays. As PCG was the predominant in-situ generated metabolite in this model, glucuronidation inhibitors (i.e. 0.75mM L-borneol, 0.075mM amentoflavone, and 0.1mM diclofenac; non-toxic conditions) were utilized to attenuate their formation, and effects on PC-associate toxicities charac-
terized. PC increased DCF formation (concentration exerting half maximal re-
sponse, EC50=0.64±0.37 mM; mean±SD, N=8), reduced GSH (EC 50=1.00±0.07 
M), and increased LDH release (EC50=0.85±0.14 mM) after 24 hrs of expo-
sure. Temporal-dependencies were evident as 1 mM PC significantly affected 
DCF and GSH markers at 6 hrs compared to LDH at 12 hrs of exposure. At 
equi-molar conditions (1 mM, 24 hrs exposure), PC was consistently more 
toxic on all markers compared to PCG and PCS. At these conditions, the intra-
cellular concentrations of PCS (7.45±1.71 M, N=3, 0.4 million cells) and 
PCG (128.45±6.14 µM) were significantly higher in cells treated exogenously 
with PCS and PCG, respectively, compared to cells treated with PC (1.39±0.09
mM for PCS and 8.1±0.25 μM for PCG). In PC- (0.75 mM for 24hours) treated cells, all three glucuronidation inhibitors selectively reduced PCG formation (without affecting PCS) by up to 56.8±22.2% (N=9) and increased PC concentrations by up to 351.09±127.78 μM. These changes were associated with further increases in LDH release by up to 346.34±173.83% (vs. PC treatment alone, N=9). Similar trends were observed for DCF and GSH markers. These novel findings indicated that PCS is the primary toxicant and that PCS and PCG are unlikely associated with PC-mediated toxicities in HepaRG cells.

2979 Evaluation of Toxic Effects of Psychoactive Substances Using Caenorhabditis elegans as a Biological Model


Econogine and delta tetrahydrocannabinol (THC) are psychoactive substances derived from the shrubs of Erythroxylum coca and Cannabis sativa, respectively. Although the consumption of these drugs has increased significantly worldwide, there are few studies related to the different effects that may cause in biota. In this work, the toxicological effects of econogine and THC were evaluated through the free-living nematode Caenorhabditis elegans, using three endpoints including mortality, locomotion, changes in the expression of genes due to the oxidative stress response (GPX-4 & SOD-4), and neural deterioration (F25b3.3). Variation in concentrations from 0.1 to 1000 μg/L of each compound were performed in the experiments. The results showed a non-significant difference in mortality, however, for locomotion endpoint a significant decrease (p<0.05) was observed for the concentration of 100 μg/L of econogine related to control. Econogine increased expression of F25b3.3, SOD-4 and GPX-4 between 4.0 and 4.5-fold greater than that in the control sample. Meanwhile, THC decreased the expression of F25b3.3 between 2 to 3.5 fold, SOD-4 from 4 to 5-fold and GPX-4 from 2 to 2.5-fold. In conclusion, econogine showed a negative effect more significant than THC, taking into account locomotion, oxidative stress & neuronal involvement of the nematode, in which the latter is being one of the main problems related to these psychoactive substances.

2980 Transcriptomic Meta-Analyses Reveal a Molecular Fingerprint Underlying Hepatic Cholestasis Response

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Cholestasis is a hepatic disease resulting from impaired bile acid metabolism and transport. Select drugs are well-established causal agents of cholestasis. Predicting the potential for a small molecule to cause cholestasis is a priority in the drug safety space due to the potential of these effects to derail development. Transcriptomic and statistical modeling approaches to identify molecular signatures of cholestasis have not yet been implemented on a wide scale. Here, we identified and integrated several public transcriptomic data sets related to cholestasis and hepatotoxicity. Briefly, 199 experimental transcriptome samples, corresponding to either control, cholestatic, or non-cholestatic hepatotoxic labels, were extracted from 8 published studies in human, rat, and mouse and from both RNA-seq and array-based platforms. Experiments included genetically, pharmacologically, and dietary induced models of disease. Differential gene expression (DGE) analysis was performed on cholestatic vs. non-cholestatic samples for 30 independent experimental contrasts to identify hallmarks of cholestatic induction. Relative expression values for 12,355 human gene orthologs were also computed and standardized in order to construct a model-based sample classifier of cholestasis. While no genes were observed as dysregulated in every cholestatic experimental contrast, 47 genes were concordantly upregulated or downregulated in at least 8 contrasts. For modeling, the gene feature space was reduced to 90 candidates through integrative analyses including DGE thresholding and Random Forest. An exhaustive Best Subset search was then employed to identify the most accurate logistic regression model for cholestatic classification, from up to 7-gene combinations validated on 70:30 training:testing data split iterations. While a random 6-gene model could be trained to classify samples with 60% accuracy, the best 6-gene model predicted cholestatic status with 84% accuracy. These studies support experimental meta-analysis as biological informative for defining cholestasis gene regulatory networks, even with diverse organisms and model systems. Ongoing work seeks to validate performance of the predictive model by independent drug treatment experiments in the HepaRG human hepatocyte model.
The zebrafish embryo model offers the possibility of studying embryogenesis on a complete vertebrate organism in an animal free regulatory context. This model is relevant to humans when studying highly conserved developmental mechanisms. One of these mechanisms is related to the retinoic acid (RA) pathway. ATRA (all-trans retinoic acid), acts as a morphogenetic regulator (MR) through regional specific expression and local concentration gradients, and interacts with other MRs like fgf8 and wnt. Together they regulate developmental processes such as neural tube formation and craniofacial morphogenesis. This makes the RA pathway a relevant target for biomarkers research in developmental toxicity studies. Establishing the optimal condition for exposure to compounds is crucial when searching for developmental toxicity biomarkers. We investigated the effects of exposure duration on gene expression in the zebrafish embryo assay. Establishing the optimal duration to anticipate malformations visible later in development. In this study zebrafish embryos were exposed shortly after fertilization to ATRA (7.5 nM) for different durations (2h, 4h, 6h, 24h, 48h, 72h, 120h), followed by transcriptomic analysis and morphological scoring. The results show an impact of the duration of exposure on both the morphological and transcriptomic readouts. Indeed ATRA induced malformations after 24h of exposure but not following a shorter exposure duration. The transcriptomic impact was observed earlier, with a larger relative difference of gene expression between 2h and 6h compared to a longer duration. For example, we observed a 2 fold increase of pxaxa, wnt3a, fgf8a and a decrease of bmp2a, pax7 and nog2. Moreover, we observed a time dependent regulation of downstream genes such as hox1b1, zic 1, fgf8. These findings highlight the relevance of the RA pathway as a source of biomarkers in developmental toxicology. These data confirm our hypothesis that transcriptomic analysis in the zebrafish embryotoxicity assay should be realized at an earlier duration than 24hpf when studying the RA pathway perturbation.

Cancer patients exhibit a broad range of inter-individual variability in both drug efficacy and drug toxicity to widely used anticancer drugs, and genetic variation is a major contributor to this variability. To identify novel genetic variants influencing drug efficacy and toxicity, we conducted a high-throughput transcriptome and genome-wide association study of 44 FDA- approved anticancer drug treatments widely used to treat various types of cancer, using 680 lymphoblastoid cell lines (LCLs) representing true global ethnic and racial diversity from the 1000 Genomes project. The drug treatments considered in this study represent nine widely used drug classes used in the treatment of cancer in addition to the paclitaxel/epirubicin combination therapy commonly used for breast cancer patients. Our GWAS found a total of 51 suggestive and significant associations. We prioritized consistent associations for functional follow-up using an integrative analysis approach which consisted of gene-expression analyses, protein QTL analysis and pathway analysis. Our results show that the NAD(P)H quinone dehydrogenase 1 (NQO1) gene is associated with the dose response of arsenic trioxide, erlotinib, trametinib, and the paclitaxel/epirubicin combination treatment. NQO1, an antioxidant enzyme important in environmental carcinoigenic detoxification, has previously been shown to be a biomarker of epirubicin response, but our results reveal novel associations with multiple anticancer drug treatments. From our integrative analyses results, we conclude that NQO1 is involved in a common drug-response pathway(s) and contributes to inter-individual variation in response to these drugs, with higher NQO1 expression and protein activity associated with increased resistance to the cytotoxic activity of these drugs. In this study, we present high-throughput in vitro screening data and genome-wide analyses results in LCLs for 44 anticancer drug treatments, including a combination treatment of paclitaxel/epirubicin. We identify multiple genetic variants associated with the response to several drug treatments. The large-scale, systematic results of this study can serve as a valuable resource for future dose-response studies for a broad range of drugs widely used for the treatment of various types of cancers.
regulated in e-cig users or smokers could help understand the metabolic diseases related to vaping and smoking. This study was supported by the National Institutes of Health (NIH) T30 HL135613, US5 CA228110, and FAMRI YFEL141014.

2987 Exploration of Cell-Free DNA in Human Embryonic Stem Cells as a Biomarker of Teratogenicity

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Chemical toxicity screening is transitioning from traditional, in vivo testing to state-of-the-art in vitro, high throughput (HTP) screening assays. Animal models for teratogen testing are both labor intensive and costly. Embryoid bodies (EBs) derived from human embryonic stem cells (hESCs) are useful as an HTP assay for testing teratogens due to their ability to differentiate spontaneously into the three germ layers. Cell-free nucleic acids are an example of molecules being investigated as emerging noninvasive biomarkers for the early detection, prognosis, prediction, and pharmacodynamic responsiveness of various diseases. The aim of this study was to evaluate if cell-free DNA (cfDNA) was released from EBs and if we could detect and explore cfDNA as a toxicological measurement in EBs derived from h9 hESCs following the exposure to known and unknown teratogenic compounds. These compounds are classified as negative, minimal, moderate, or high-risk teratogens by the Teratogen Information System (TERIS). Following a 10-day exposure to the compounds, the supernatant was collected and cfDNA was extracted. The total cfDNA concentration for stem EBs, lysine serum, O TA, u fragment, and uracilized. Known teratogens showed higher cfDNA concentrations compared to non-teratogens and vehicle controls. In addition, the cfDNA isolated from EB supernatants were analyzed in other molecular assays such as droplet digital PCR (ddPCR). Sequencing of cfDNA is being considered as a next step for selected teratogens. This study demonstrated cfDNA as a potential biomarker and useful tool for high-throughput screening of teratogenic compounds in state-of-the-art, in vitro toxicology prediction studies.

2988 Mycotoxins Exposure in Children Resided in Banke Region, Nepal

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Dietary mycotoxin contamination has been a major public health issue for decades, particularly in the developing world. The most prominent of which are aflatoxins, a group 1 carcinogen known for their immunosuppressive, hepatotoxic and growth inhibitory effects on animals, and their role as a major risk factor for liver cancer and childhood stunting in many low- and middle-income countries. Recent studies have shown that co-exposure with other mycotoxins may have interactions, including synergistic effects on the toxicity. Nepal has a high stunting rate with a prevalence of 36%, and recent reports demonstrate the immense potential of utilizing lung tissue-derived EVs for diagnostic and prognostic biomarker for chronic lung diseases. Furthermore, EVs belong to allograft rejection, asthma and autoimmune thyroid diseases. The composition of the EVs largely depends on the pathophysiological state and also on the cell type of origin like alveolar macrophages, neutrophils, eosinophils, dendritic cells, the alveolar and airway epithelium. Isolation and characterization of EVs from bronchoalveolar lavage fluid and serum from patients with chronic lung diseases using next-generation sequencing approaches revealed useful biomarkers previously. We hypothesize that lung tissue-derived EVs from allergen-exposed mice contain highly enriched protein cargo that may be used as novel protein biomarker in asthma. C57BL/6J mice were either exposed to house dust mite (HDM, 30 μg) or phosphate-buffered saline (PBS) for 10 consecutive days and lung tissue was harvested 48 hours post-last exposure. EVs were isolated from the lung tissues via ultracentrifugation and characterized using NanoSight NS300 and transmission electron microscopy (TEM). The surface marker of the EVs were characterized by immunoblotting. Additionally, the lung tissue-derived EV cargo were analyzed by high-resolution mass spectrometry (MS) using label-free quantification. Isolated EVs were significantly enriched in the allergen-exposed (HDM) group compared to the control (PBS) group as confirmed by nanoparticle tracking analysis, TEM, and immunodetection of EV surface markers such as flotillin-1 and caveolin. MS analysis revealed 665 proteins with a fold-change above 1.5 or below 0.1-1.5 with quantitative ratios considered as significant. Cluster analysis of the differentially enriched EVs proteins revealed 35 proteins increased and 85 proteins decreased significantly (P < 0.05) in the HDM group compared to control. Proteins identified were functionally annotated by GO, KOG and COG, and protein-protein interaction was also conducted. KEGG pathway analysis revealed top 3 novel pathways enriched in identified differentially enriched proteins. These proteins belong to allograft rejection, asthma and autoimmune thyroid diseases. Abundant EVs proteins were also validated by immunoblotting. Our study demonstrates the immense potential of utilizing lung tissue-derived EVs for a better understanding of the pathophysiology of asthma, and as a novel diagnostic and prognostic biomarker for chronic lung diseases. Furthermore, Propylene oxide (PO) is a reactive industrial chemical that induces nasal tumors in rodents following chronic high-dose inhalation exposures. The cellular key events (KEs) of a nonmutagenic MOA for PO-induced nasal tumors have been proposed; however, detailed mutation analysis gaps have remained unresolved. This study will evaluate the association between mycotoxin exposure levels and child health outcomes. Analyses will be conducted to evaluate the association between mycotoxin exposure levels and child health outcomes.
Dopamine (DA) homeostasis disruption is a hallmark of neurodegenerative disorders such as Parkinson’s disease (PD). DA is metabolized by monoamine oxidase to 3,4-dihydroxyphenylacetaldehyde (DOPAL), a biogenic aldehyde known to be highly reactive and cytotoxic. The “catecholaldehyde hypothesis” states that DOPAL and other biogenic aldehydes are toxic to cells and contribute to cell death. The disruption of DA homeostasis, and consequent DOPAL accumulation, is likely part of the greater etiopathology of PD. PD is characterized by death of dopaminergic neurons in the substantia nigra, but this disease is not clinically diagnosed until patients exhibit motor symptoms, although mechanisms of etiopathology (i.e. DA homeostasis disruption) begin many years before these symptoms’ onset. Our lab has found a novel biomarker that may mark dopamine dyshomeostasis and therefore could be useful for diagnosing neurodegenerative disorders. We have found that DOPAL reacts with the amino acid L-cysteine to form a putative thiazolinedione conjugate that is identifiable using mass spectrometric methods. This conjugate (DOPAL-cys) forms in vitro in SH-SYSY cells after treatment with 100µM DA and 5mM N-acetyl cysteine. Conjugate production is not observed when only treated with DA, and conjugate production is diminished when also treated with monoamine oxidase inhibitors. Remarkably, SH-SYSY cells also produce DOPAL-cys when treated with 100µM DA and 1µM rotenone, a pesticide with epidemiological and mechanistic implications for PD. Collectively, these experiments show that SH-SYSY cells produce DOPAL-cys when 1) under conditions of excess precursors to DOPAL and L-cysteine or 2) under conditions of stress when DOPAL is also present. We have also found that this conjugate is transported extracellularly after production.

Ethyl glucuronide (EtG) is a minor non-oxidative conjugated ethanol metabolite formed in low amount after alcohol consumption. Compared with breath ethanol, EtG is excreted in urine for a prolonged time. This study compared the performance of breath ethanol and urinary EtG with alcohol use in heavy drinkers. Using cross-sectional study design, one hundred and twenty two alcohol dependent patients (diagnosed as per International Classification of Diseases, Version-10) with last alcohol consumption within 24 hours were recruited after their consent. The subjective information included: socio-demographic details, alcohol use details and alcohol amount consumed in past three months (by beverage-specific quantity-frequency method). Breath test was done on spot with the help of a breath analyser. Urine was collected to assess for EtG. All subjects provided a urine sample within 24 hours of breath test. The mean age of the participants was 37.7 (7.6) years. All participants used alcohol daily, locally brewed liquor being the preferred beverage (56%). The mean age of onset of daily alcohol consumption was 27 (SD:6.2) years and the mean age of onset of early morning drinking was 30.5 (SD:9.8) years. Mean quantity of alcohol consumed before admission was 103.13 (47.21) grams per person. EtG values expressed a strong correlation (r = 0.801, p < 0.001) with quantity of alcohol consumed. Urinary EtG showed 95% sensitivity (95% CI: 76.8, 97.8) with respect to last dose of alcohol consumed while breath ethanol showed 86% sensitivity (95% CI: 75.6,93.5). The area under the curve (AUC) by receiver operating characteristics (ROC) analysis to distinguish heavy drinkers from moderate drinkers was 0.98 (95% CI: 0.95, 1.0) for urinary EtG and 0.43 (95% CI: 0.33, 0.54) for breath ethanol. The study showed that urinary EtG showed an optimum diagnostic performance to detect alcohol intake in heavy drinkers in the previous 24 hours.
Vaccines provide a crucial platform in the fight against infectious diseases by priming the adaptive immune system to elicit prompt, specific, and effective cell and humoral-mediated responses to eliminate pathogens. The development of a vaccine requires thorough assessment of both efficacy and safety, particularly with respect to the severity of immediate immune activation effects, or reactogenicity. Highly reactogenic vaccines have the potential to induce an overwhelming inflammatory response which can lead to critical illness and multi-organ failure in patients. In vitro measurements are taken at the preclinical stage to gain insight into the multi-parametric dynamics of inflammatory responses and to avoid severe adverse side effects at successive clinical phases. An important, open question in the field of vaccine safety is how to translate in vitro assays to accurately assess vaccine-induced reactions in human clinical trials. The Peripheral Tissue Equivalent (PTE) module of the MIMIC® in vitro system is designed to recapitulate the innate immune response to challenge. Within the PTE module, monocytes autonomously differentiate into heterogeneous dendritic cell populations without exogenous factors, thereby closely approximating physiological conditions. Using this construct, we explored how 14 vaccines of varying reactogenicity at several dilution factors impact cytokine levels. 12 individual cytokines and 5 flow cytometry parameters were measured across 40 donors. We applied various informatics approaches to produce a relative vaccine reactogenicity order. A combination of hierarchical clustering, principal component analysis, and feature selection suggest only a subset of measurements may be needed to assess a vaccine’s in vitro reactogenicity. Next, we used publicly available clinical data from patients treated with the same vaccines to construct a scoring framework. The score estimates a treatment’s clinical reactogenicity range by considering the frequency and severity of observed local and systemic adverse effects. Pairwise combination analysis was performed to determine which subset of adverse effects can effectively discriminate treatments. Taken together, we will develop a quantitative systems toxicology model that translates in vitro PTE data to accurately assess the risk of severe adverse effects for vaccines and other biologic drugs.

microRNA-122 (miR-122) is a circulating, liver-specific biomarker of drug-induced liver injury (DILI). miRs can exist in multiple isoforms (isoforms) that vary in length as demonstrated by small RNA sequencing. Our aim was to determine the tissue distribution of miR-122 by using a mouse model of acetaminophen (APAP)-induced liver injury; to profile isoforms of miR-122 in man; and to utilize a novel assay (3′-Db-PCR) to selectively quantify a DILI-specific isoform of miR-122. Male C57BL/6J mice were treated intraperitoneally with APAP (300mg/kg) or vehicle only and monitored for 6-12h. miR-122 expression in the blood and major organs was determined by PCR. The expression of miR-122 isoforms in man was determined by small RNA sequencing. Synthesized canonical miR-122 and 3′-iso-miR-122 were used in an off-the-shelf SYBR green-based and TaqMan-based assays. The 3′-Db-PCR assay was used to selectively quantify a DILI-specific miR-122 3′-isoform. Following 6h APAP treatment, liver miR-122 expression decreased and circulating miR-122 significantly increased to a mean of 2.82x10⁶ copies/µL (95% CI [1.07x10⁶, 4.38x10⁶], P<0.0001). At 12h following treatment, circulating miR-122 had decreased toward control levels. miR-122 significantly increased in the renal cortex, renal medulla and spleen following APAP treatment. Small RNA sequencing demonstrated various miR-122 isoforms with 3′-variants being highest in abundance. The efficiency of ‘off-the-shelf’ assays progressively decreased as 3′-nucleotides were removed from miR-122. Cycle threshold (Ct) values obtained following 3′-Db-PCR of mouse plasma in a number of studies following APAP treatment with mean Ct of 23.0 (95% CI [21.7, 24.3], P<0.0001) when compared to the control mean of 31.0 (95% CI [27.9, 34.1]). In the renal cortex, Cts were significantly lower following APAP treatment at both 6h with a mean Cc of 30.7 (95% CI [29.8, 31.5], P=0.006) and 12h with a mean Cc of 33.0 (95% CI [31.5, 34.5], P=0.028) when compared to controls. Cts were also significantly lower in the spleen in APAP-treated mice at both 6h with a mean Cc of 29.6 (95% CI [29.1, 30.2], P<0.0001) and 12h with a mean Cc of 31.7 (95% CI [30.4, 33.0], P=0.032) when compared to controls. No significant changes were observed in the liver. In summary, APAP-DILI leads to the release of various miR-122 isoforms into the circulation, which are cleared by the kidney and spleen.

The Health Opportunity Index (HOI) is a multivariate tool that can be more efficiently used to identify and understand the interplay of complex social determinants of health (SDH). The census tract level influences the ability to achieve optimal health. The derivation of the HOI utilizes the data-reduction technique of principal component analysis to determine the impact of SDH on optimal health at lower census geographies. In the midst of persistent health disparities and the present COVID-19 pandemic, we demonstrate the potential utility of using 13 input variables to derive a composite metric of health (HOI) score as a means to assist in the identification of the most vulnerable communities during the current pandemic. Using GIS mapping technology, health opportunity indices were layered by counties in Ohio to highlight differences by census tract. Collectively we demonstrate that our HOI framework, principal component analysis and convergence analysis methodology in combination with the Public Health Exposure Framework, coalesce to provide results supporting the utility of this framework in the three largest counties in Ohio: Franklin (Columbus), Cuyahoga (Cleveland), and Hamilton (Cincinnati). The results in this study identified census tracts that were also synonymous with communities that were at risk for disparate COVID-19 related health outcomes. In this regard, convergence analyses facilitated identification of census tracts where different disparate health outcomes co-exist at the worst levels. Our results suggest that effective use of the HOI composite score and subcomponent scores to identify specific SDH can guide mitigation/intervention practices, thus creating the potential for better targeting of prevention and intervention strategies for vulnerable communities, such as during the current pandemic.
Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19). As of November 17, 2020, more than 55.4 million confirmed cases and 1.3 million deaths have been reported. Thus, it is immensely important to develop drugs and vaccines to combat COVID-19. The spike protein present on the outer surface of the virion plays a major role in viral infection by binding to receptor proteins present on the outer membrane of cells, triggering membrane fusion and internalization which enables release of viral sRNA into the host cell. Understanding the interactions between the SARS-CoV-2 trimeric spike protein and its host cell receptor protein, angiotensin converting enzyme 2 (ACE2), is important for developing drugs and vaccines to prevent and treat COVID-19. Several crystal structures of partial and full-length SARS-CoV-2 spike protein have been reported; however, an atomic structure of the wild-type SARS-CoV-2 trimeric spike protein complexed with ACE2 is not yet available. Therefore, in our study, homology modeling was used to build the trimeric form of the spike protein complexed with human ACE2, followed by all-atom molecular dynamics simulations to elucidate interactions at the interface between the spike protein and ACE2. Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) and in silico alanine scanning were employed to characterize the interacting residues at the interface. Twenty interacting residues in the spike protein were identified that are likely to be responsible for tightly binding to ACE2, of which five residues (Val445, Thr478, Gly485, Phe490, and Ser494) were not reported in the crystal structure of the truncated spike protein receptor binding domain (RBD) complexed with ACE2. These data indicate that the interactions between ACE2 and the tertiary structure of the full-length spike protein trimer are different from those between ACE2 and the truncated monomer of the spike protein RBD. These findings could facilitate the development of drugs and vaccines to prevent SARS-CoV-2 infection and combat COVID-19.
Interaction between COVID-19 infection and chemical exposures is an emerging public health concern. Air pollution is responsible for >15% increase of death rate from COVID-19. Epidemic itself significantly increases exposures to cleaning and disinfectant product. Mechanisms of interaction between COVID-19 and variety of chemicals is yet unexplored. To identify possible synergism in the gene expression affected by chemicals and COVID-19 using in-silico approach. Data on 2169 high-throughput toxicological studies were extracted from the Comparative Toxicogenomic Database and sensitivity of each gene to chemical exposures was identified as a number of chemical-gene interactions. Normalizing microarray gene expression values for different human cells and tissues were obtained from the Human Protein Atlas. Coronascape tool of Metascape analysis was used to check if genes responsive to COVID-19 in different human cells are enriched with cell-specific genes highly sensitive to chemical exposures. We have found significant overlap in immune response genes activated in several cell-types in response to COVID-19 and activated in response to a broad range of chemical exposures. Our data identify synergy in gene expression changes induced by chemical exposures and COVID-19. This synergy may explain higher risk of severe symptoms and death among COVID-19 patients chronically exposed to high doses of environmental, occupational and other pollutants. This study can serve as a guide to future mechanistic studies geared towards understanding the relationship between chemical exposure and COVID-19.

Modelling COVID-19 Pathogenesis Using the Adverse Outcome Pathway Framework: The CIAO Project

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The coronavirus disease (COVID-19) has been declared a global pandemic by the World Health Organization. Researchers around the world have mobilized to study the SARS-CoV-2 virus and the resulting disease, generating a flood of biological data from a wide range of disciplines. COVID-19 has been linked to many adverse health outcomes, including myocarditis, acute respiratory distress, and deep vein thrombosis. To resolve ambiguity in the COVID-19 disease process, there is a need for an organized data structure to assist scientists in understanding how COVID-19 affects the many population groups that have been impacted by this disease. The Adverse Outcome Pathway (AOP) framework is an ideal structure for this task. An interdisciplinary project called CIAO (Modelling the pathogenesis of COVID-19 using the Adverse Outcome Pathway framework) has been initiated by the European Commission’s Joint Research Centre to address this need. One goal of the CIAO project is to assist researchers in identifying likely adverse outcomes by SARS-CoV-2 infection that can result from a patient’s clinical history to help direct individualized treatment. As a pilot effort, we focus specifically on inflammation in COVID-19, as part of the Bradykinin pathway. Established information in the EPA Adverse Outcome Pathway Database (AOP-DB; aopdb.epa.gov) and WikiPathways (wikipathways.org) describe the molecular basis for Bradykinin-induced inflammation and activation by metal and metal oxide nanomaterial exposure, such as titanium dioxide (TiO2). Exposure to TiO2 augments production of reactive oxygen species, which leads to oxidative stress in humans and up-regulates inflammatory biomarkers associated with adverse cardiovascular outcomes. Insight into AOPs related to TiO2 exposure, inflammation and CVD related disease may be helpful to understand the increased severity of COVID-19 response in geographic areas with high levels of air pollution. This abstract does not reflect US EPA Policy.

Assessing the Risk of Coronavirus Transmission in Different Counties of Kansas and Identifying the Relative Impact of Environmental Factors and Human Demography

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The 2019 coronavirus disease (COVID-19) is an emerging infectious disease and has developed into a global pandemic. It is crucial to assess the risk of spatial spreading of COVID-19 and devise effective mitigation strategies since reopening became necessary after the lockdown. The necessity to identify high-risk individuals became exponentially important during the reopening. As human contacts and movement patterns play a significant role in the spread of COVID-19, network-based models can capture the transmission dynamics of COVID-19. These network-based models can be used for COVID-19 trans...
mission risk assessment and identify the impact of different factors such as the contact and mobility pattern and environmental factors such as temperature and rainfall. This study aimed to develop a risk assessment framework for COVID-19 transmission and to identify the relative impact of human demographics (contacts and movement patterns) and environmental factors. The application of the framework was demonstrated with an application for COVID-19 transmission in Kansas. We developed a spatially explicit human contact network for Kansas, where each spatial unit was a county. The movement pattern was incorporated in the contact network so that counties with big cities and ample educational facilities would have numerous connections with the rest of the state. We performed simulations using an eight-compartment modified Susceptible-Exposed-Infected-Recovered (SEIR) model. Two scenarios were considered: pathogen transmission was dependent on the environmental factors and did not depend on the environmental factors. Preliminary simulation results showed that the contact and movement patterns dominated the overall transmission dynamics of COVID-19. Incorporation of the climate data into the model and developing the risk maps are still ongoing. This model provides a computational framework to assess the impact of environmental factors and human demographics on the transmission of COVID-19 and can be extrapolated to other States and potentially the entire U.S.

3008 Risk and Protective Factors in the COVID-19 Pandemic: A Rapid Evidence Map (rEM)

Given the widespread spread of the 2019 Novel Coronavirus (COVID-19), there is an urgent need to identify risk and protective factors and expose areas of insufficient understanding. Emerging tools, such as the Rapid Evidence Map (rEM), are being developed to systematically characterize large collections of scientific literature. As the number of COVID-19 studies being published on a daily basis continues to grow exponentially, the ability to efficiently synthesize size knowledge that could be used to improve public health is paramount. In our rEM, utilizing previously established methods and structured approach to identify risk and protective factors for COVID-19, our rEM identified promising research areas such as age, gender, comorbidity association with COVID-19 and supporting literature to potentially inform a follow-up review, such as a systematic review, and other areas e.g., risk factors in susceptible sub-groups, and risk factors in children where evidence is lacking and further research may be necessary. Depending on the scope of the question being addressed, the time required to complete a typical rEM can be measured in weeks or months compared to systematic reviews, which often take 1-2 years or even longer. Our rEM, developed within one calendar month, represents a useful tool for quickly synthesizing literature on a particular topic of interest and identifying data-rich/data-poor areas within the topic on hand that may benefit from additional investigation. The automation technologies employed can also be used to periodically update the resulting evidence base and track scientific knowledge as it evolves, providing an informative tool for researchers to stay up-to-date on quickly-evolving topics and rapidly-growing bodies of literature. The rEM methodology is a valuable tool that can be applied to a wide range of topics in clinical, environmental health, and related scientific disciplines. Furthermore, since rEMs make substantial use of machine learning and information retrieval applications and software, continued development and expansion of the capacity of such tools is likely to further enhance the capacity and accuracy of rEMs.

3009 Dysregulated ACE2 Activity and Cytokine Profile amongst Smokers Could Increase Disease Susceptibility toward SARS-CoV2

COVID-19 is caused by the SARS-CoV2 virus, leading to acute lung injury and lung remodeling in the airway and alveolar regions of the lungs, which eventually culminates to lung pneumonia. Tobacco smoking is known to cause lung oxidative stress and inflammatory responses, rendering susceptible to infectious agents. However, the progression of lung injury based on disease susceptibility and severity amongst smokers and people with pre-existing lung conditions e.g. compromised lung function and immunity is not known. We hypothesized that dysregulated COVID-19 receptor, ACE2, expression and activation by smoking-induced immune response would result in severe disease outcomes amongst smokers. To test our hypothesis, we performed in vivo experiments on CS-exposed young and old C57BL/6J mice and found age-in-
dependent increase in the expression of ACE2 in the lungs from CS-exposed mice as compared to air controls. Further, we obtained serum from COVID-19 positive and COVID-19 recovered patients with and without a smoking history and studied the ACE2 activity by enzymatic assay. There was a significant dysregulation in the ACE2 activity of COVID-19 positive patients as compared to COVID-19 recovered patient group. Smoking habit was found to affect the ACE2 activity in COVID-19 positive group. Additionally, there was a significant gender-based variation in the ACE2 activity amongst COVID-19 positive patients. Further, a distinct cytokine profile amongst COVID-19 positive patients with a smoking history as compared to the non-smoking controls was observed. Overall, these data suggest that the onset and progression of COVID-19 varies amongst smokers and non-smokers. Further work is in progress to determine the role of smoking in the disease pathogenesis of COVID-19.

3010 Evaluating Consumer Exposure to Disinfecting Chemicals against Coronavirus Disease 2019 (COVID-19) and Associated Health Risks
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Disinfection of surfaces has been recommended as one of the most effective ways to combat the spread of novel coronavirus (SARS-CoV-2) that causes coronavirus disease 2019 (COVID-19). However, overexposure to disinfecting chemicals may lead to increased health risks. Using an indoor fate and chemical exposure model, we estimate human exposure to 22 disinfecting chemicals on the lists recommended by various governmental agencies against COVID-19, resulting from contact with disinfectated surfaces and handwashing. Three near-field exposure routes, i.e., directly- and mouth-mediated oral ingestion, inhalation, and dermal absorption, are considered to calculate the whole-body uptake doses and blood concentrations caused by single use per day for three age groups (3, 14, and 24-year-old). We also assess the health risks by comparing the predicted whole-body uptake doses with in vivo toxicological data and the predicted blood concentrations with in vitro bioactivity data. Our results indicate that the predicted relative contribution from each exposure route vary considerably among the disinfecting chemicals due to their diverse physicochemical properties. 3-year-old children have consistent higher exposure than other age groups, especially in the scenario of contact with disinfectated surfaces, due to their more frequent hand contact and mouthing activities. Due to the short duration of handwashing, we do not expect any health risk from the use of disinfecting chemicals in handwashing. In contrast, exposure from contact with disinfected surfaces may result in health risks for certain age groups especially children, even the surfaces are disinfectated once a day. Interestingly, risk assessments based on whole-body uptake doses and in vivo toxicological data tend to give higher risk estimates than do those based on blood concentrations and in vitro bioactivity data. Our results reveal the most important exposure routes for disinfecting chemicals used in the indoor environment; they also highlight the need for more accurate data for both chemical properties and toxicity to better understand the risks associated with the increased use of disinfecting chemicals in the pandemic.

3012 Exhaled Aerosols and Transmission of Particles Presumed to Contain Respiratory Viruses: An Observational Study in Normal, Healthy, as Well Clinically Ill Persons
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The transmission of respiratory viruses such as influenza and coronavirus viruses from one person to another is still not fully understood. Reports are found in the literature showing release of submicron particles (Fabian et al. JAMPPD, 24(3) 137-147, 2011. Thus, there is already evidence that, at least for Rhinoviruses, these are very efficiently spread via aerosols generated by a person or by a patient’s breathing. It is further necessary for the patient to cough or sneeze. The exhaled aerosol particles are generated by normal respiration and airway within the deep lungs, via reopening of collapsed small airways between inspiration and expiration, and act as a particle source. These mucous/surfactant aerosols (size range between 0.2 - 0.6 µm) can transport viruses out of the lungs of patients and then, based on size considerations, may be present in the exhaled air within a room, possibly for hours. A measuring system was designed which consists of an optical particle spectrometer and a special mouthpiece with an absolute sub-micron filter. A subject inhaling through
The filter prevents ambient aerosol particles from entering the lungs and then, after about 5 - 10 cleansing breaths, the exhaled particles detected by the system must have been generated within the lungs. We have measured more than 200 healthy as well as sick individuals. The particles that are generated within the lungs are usually between 0.2 and 0.4 μm in diameter. The number concentration for healthy individuals is between a few - 2000 particles / L. The average particle concentration for currently marketed medical device components/materials was found to reach 40,000 - 125,000 particles per L. Additional research and data, currently being gathered, will be presented.

3013 Dysregulation in microRNA Expression in Peripheral Blood Mononuclear Cells from Patients with COVID-19

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The coronavirus disease-2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). COVID-19 has triggered a pandemic and major public health issue worldwide nowadays. Studies suggest that the immune responses may play a critical role in COVID-19 pathogenesis. Epigenetic mechanisms such as induction of microRNAs (miRNAs) that are independent of genomic approaches have recently been shown to play a major role in immune activity, and consequently development and progression of the disease. Therefore, change of miRNA expression may play an important role in the pathogenesis of COVID-19. In the current study, we investigated the induction of miRNAs during SARS-CoV-2 infection and their role in the regulation of inflammation. Figure 1 illustrated that COVID-19 had significant increased numbers of peripheral blood mononuclear cells (PBMCs) in the peripheral blood samples as compared with healthy controls. Sequencing analysis of miRNA expression revealed that 50 miRNAs such as hsa-miR-150-5p, hsa-miR-342-3p, hsa-miR-495-3p, hsa-miR-409-3p and hsa-miR-146b-5p were significantly down-regulated in PBMCs from patients with COVID-19, whereas 48 miRNAs including hsa-miR-223-5p, hsa-miR-650, hsa-miR-450a-5p, hsa-miR-6502-5p, hsa-miR-144-3p, hsa-miR-3960 and hsa-miR-148a-3p were significantly up-regulated in comparison with those from healthy controls. Analysis of miRNA functions and their roles in diseases showed that dysregulated miR-143-3p, miR-145-5p, miR-146a-5p and miR-148a-3p could regulate cell growth and proliferation. Dysregulated miR-424-3p, miR-450a-5p, miR-451a and miR-487b-3p could control cell survival and death. Dysregulated miR-150-5p, miR-223-3p, miR-27a-3p, miR-403-5p and miR-941 could regulate inflammatory responses. Dysregulated miR-145-5p, miR-154-3p, miR-16-5p and miR-874-3p could be responsible for psychological disorders in patients with COVID-19. Particularly, down-regulated miR-150-5p could target Akt signaling pathway and thus increase cell growth and proliferation of PBMCs in patients with COVID-19. Up-regulated miR-16-5p and miR-424-3p could target Checkpoint kinase 1 (CHEK1), an inducer of cell cycle arrest, and thus promote cell cycle. Down-regulated miR-146a-5p targeted IL36 and IL37, and thus activated T cell proliferation and induced the release of IL-2, which might be responsible for the increased numbers of PBMCs in patients with COVID-19. Our analysis uncovered the immunological associations of dysregulated miRNAs with clinical disease. The current study demonstrates that miRNA dysregulation correlates with clinical manifestations and inflammation, and therefore specific miRNAs may represent potential therapeutic targets against COVID-19. Supported by NIH grants P01AT003961, P20GM103641, R01AT06888, R01MH094755, R01AI29788 and R01AI123947.

3014 The Suitability of Reconstructed Human Epidermis Models for Medical Device Irritation Assessment: A Comparison of In Vitro and In Vivo Testing Results


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The ISO 10993 standards on biocompatibility assessment of medical devices discourage the use of animal tests when reliable and validated in vitro methods are available. A round robin validation study of in vitro reconstructed human epidermis (RHE) assays was conducted to evaluate their potential as replacements for the rabbit skin patch test. The round robin determined that RHE assays were able to correctly identify strong irritants in dilute medical device extracts. However, there was some uncertainty about whether RHE tissues accurately predicted the results of the rabbit skin patch test and the intracutaneous irritation test. To address that question, this poster presents in vivo data from the round robin and subsequent follow-up studies. The follow-up studies included simultaneous in vitro RHE model and in vivo testing of round robin polymer samples and the results of dual in vivo/in vitro testing of currently marketed medical device components/materials. Our results show for the first time that for both pure chemicals and medical device extracts, the intracutaneous rabbit test is more sensitive for detecting irritant activity than the rabbit skin patch test. The studies also showed that the RHE models produced results that were essentially equivalent to those from the intracutaneous rabbit skin test for acute contact dermatitis. Therefore, RHE in vitro models are acceptable replacements for the in vivo rabbit intracutaneous irritation test for evaluating the irritant potential of medical devices.

3015 Accelerated Method to Predict Long-Term Nickel Release from Nitinol


Nitinol is commonly used in medical devices because of its unique shape memory and pseudoelasticity properties. However, nitinol is also known to release nickel from its surface, and this can lead to local and systemic toxicity concerns in patients. Therefore, nickel release from nitinol-based devices is typically evaluated by chemical-specific data or a suitable analogue for read-across, the POD is assigned. For identifying an appropriate point of departure (POD). In the absence of chemical-specific data or a suitable analogue for read-across, the POD is typically characterized through time-consuming activity concerns in patients. Therefore, nickel release from nitinol-based devices is typically characterized through time-consuming tests to generate nickel exposure estimates. The medical device community can benefit from methods that accelerate this type of testing from 60 days to much shorter periods. In this study we explore use of elevated temperature (above 37 °C) as a potential method to expedite this testing. Nickel release from straight nitinol wires finished chemically (etched, chemical-etched, amber oxide and black oxide) was characterized at four different temperatures (37 °C, 52 °C, 67 °C and 87 °C). The nickel surface finish spanned a range of thin oxide (similar to finished medical devices) to thicker oxide, which model worst-case finishes. Nickel release was quantified using inductively-coupled plasma mass spectrometry. Pitting corrosion susceptibility was assessed using ASTM F2129 and aerosol spectroscopy was used to characterize elemental compositional depth profiles and oxide thickness. We found that for three of the materials with relatively thin oxide layers (electropolished, chemical-etch and amber-oxide), nickel release exhibited Arrenius behavior over the entire temperature range. However, nickel release from black oxide nitinol, which had a much thicker oxide, did not follow Arrenius behavior. To illustrate the potential benefit of using elevated temperature to abbreviate nickel release testing for estimating exposure, we conducted an expedited study that showed a greater than 50-day nickel release profile at 37 °C could be accurately recovered by testing for less than one week at 67 °C. To address precision and bias, and to account for other device configurations, finishes, and release profiles, additional testing is needed to establish a protective temperature scaling that can be routinely used to evaluate nickel release from all nitinol based medical device components.

3016 Applicability of the European Chemicals Agency (ECHA) Database in Medical Device Toxicological Risk Assessments (TRAs)


Medical device toxicological risk assessments (TRAs) apply a tiered process for identifying an appropriate point of departure (POD). In the absence of chemical-specific data or a suitable analogue for read-across, the POD is based on the evaluation of the chemical structure through (Q)SAR tools, and assignment of Threshold of Toxicological Concern (TTC), when appropriate. Consistent with ISO/TS 21726 (2019), application of the TTC is most appropriate for scenarios in which chemical-specific or appropriate surrogate data do not exist. However, some common medical device extractables, including polymeric fragments and oligomers, are excluded from the use of TTC, highlighting the need for consideration of all available toxicological sources. Recent regulatory feedback has indicated that in the absence of a peer-reviewed publication, the POD should default to the TTC, precluding the use of data from sources such as the European Chemicals Agency (ECHA) database. The ECHA database is an invaluable source of toxicological data, with more than 23,000 unique registered chemicals in the database as of November 2020. The study details are publicly available, and unlike many peer-reviewed publications, they are scored for reliability, predominantly GLP compliant, and conducted in accordance with OECD test guidelines. As such, these studies are ideal for toxicological risk assessment as they meet the necessary criteria for
hazard identification and dose-response evaluations. Use of such data is also consistent with ISO 10993-1 (2018), which states that “all available unpublished relevant data should be taken into account in order to avoid publication bias.” Limiting medical device TRAs to only peer-reviewed publications or TTC values, if appropriate for non-excluded compounds, could result in either under or over-estimation of potential risk. To demonstrate the importance of considering data from ECHA in the TRA process, Margins of Safety (MOS) for both short- and long-term exposure scenarios were calculated using toxicology data available only in ECHA versus defaulting to a TTC. In general, defaulting to a TTC resulted in substantially lower MOS (i.e., overestimation of risk) when compared to the derived tolerable intake (TI) using experimental data from ECHA. This presentation highlights the importance of considering all available toxicological data for medical device TRAs in order to appropriately characterize potential patient risk and avoid publication bias.

3018 Optimization of Extraction Method B Using Common Organic Solvents for Dosing in Genotoxicity In Vitro Assays

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Medical devices are a wide range of instruments used to treat, prevent, mitigate or aid the diagnosis of human health. The complexity of these devices increases upon patient needs, raising also safety concerns. Among the important end points for hazard identification is genotoxicity. ISO 10993-3 requires to use three methods for sample preparation. These are called Method A, B and C and their formulation concentrations decrease going from a to c. To use Method A requires being able to dissolve or resuspend the material in a vehicle that is compatible with the test system. If dissolving or resuspending the device is not possible, Method B can be used. This involves mixing the device in a solvent for 24 hours, followed by evaporating the solvent, and weighing the residues. If there is a sufficient amount of residues obtained, this method can then be used to prepare the sample for the main study. Otherwise, the device will need to be extracted as per Method C in a polar or non-polar vehicle that is compatible with the test system. Therefore, choosing the appropriate method for sample preparation is critical, with Method B being the most challenging. Often, there is also a limiting number of devices available, and amount of residue that can be extracted, making it difficult to select the appropriate method for the main test. The objective of this investigation was to determine the maximum feasible dosing volume of several common solvents that, when used in the Method B protocol, would produce higher concentrations when residues were resuspended in smaller final volumes. The experiment was performed with the bacterial reverse mutation (Ames) test using plate incorporation and pre-incubation methods, and with the in vitro micronucleus (IVM) test using human peripheral blood lymphocyte cells. The solvents tested were methanol, ethanol, acetone and hexane. The results indicated that cytotoxicity limits the use of solvents at high volumes. In the Ames test, the solvents were more toxic in the pre-incubation method, and the strains had different sensitivity. Overall, acetone, methanol and ethanol could be administered at 50 µL/plate, and hexane at 25 µL/plate. In the IVM test, the solvents were more toxic in 24 hours treatment period. The ethanol and methanol could be administered at allowable limit of 10 µL/mL while ethanol and hexane could be administered at 5 µL/mL. These volumes are considered satisfactory, as they allow higher dosing concentrations to be achieved whenever Method B is chosen for sample preparation.

3019 How Sensitive Should Chemical Characterization of Medical Devices Be? Calibration of Analytical Evaluation Thresholds with the Carcinogenic Potency Database

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Chemical characterization is an essential component of the safety evaluation of medical devices and combination products. An analytical evaluation threshold (AET) derived from a Threshold of Toxicological Concern (TTC) is recommended to calculate the required analytical sensitivity for a screening chemical analysis. There is a lack of consensus whether to use 1.5 or 120 µg/day in calculating the AET for long-term contact medical devices, with the lower value sometimes requiring sensitivities beyond current analytical capabilities or the manipulation of extracts. The Carcinogenic Potency Database (CPDB) was reviewed to compare the risks associated with using either value to calculate the AET. The oral route of GARD™ were CPDB was used to identify substances with TD50s. The corresponding TD50s from the Lhasa CPDB for non-Cohort of Concern (non-COC) substances were multiplied by the corresponding exposure durations to calculate the total doses. Total doses were linearly extrapolated to an excess cancer risk of 10⁻⁶. The number of non-COC substances that would exceed this risk using either AET was nine with 1.5 µg/day or 120 µg/day were then compared. From the 199 substances evaluated, only two posed an excess risk at an AET calculated with 1.5 µg/day and only nine with 120 µg/day. Furthermore, over 95% of non-COC substances would not pose an excess cancer risk using an AET calculated with 120 µg/day. Because only perhaps 10 percent of chemicals are carcinogens and even less are genotoxic carcinogens, the probability of a “new” medical device extractable posing a risk greater than 10⁻⁶ at 120 µg/day is less than 0.5 percent. Based on our evaluation, an AET based on 120 µg/day is protective and practical for chemical characterization of medical devices. Use of such an AET will allow toxicological risk assessments to focus on those extractables that potentially pose the greatest risk to patients.

3020 Applicability Domain of the GARDskin Medical Device Test for In Vitro Skin Sensitization Testing of Medical Devices

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Medical device toxicology is undergoing an exciting evolution; transitioning from a process that largely relied on the results of animal testing to evaluate the biological safety of devices in patients to one which is increasingly focused on the use of in vitro methods for the safety assessment of device materials. Recently, in vitro methods to assess endpoints such as skin irritation and pyrogenicity have been validated and proposed for medical device testing, but a method to assess the potential for device-related skin sensitization to occur has not been sufficiently qualified. A number of in vitro skin sensitization test methods have been shown to have acceptable predictive ability for known skin sensitizers with structures that span a broad range of chemical classes, but the predictive ability of these methods has not been specifically evaluated using compounds typically found in materials used to manufacture medical devices. As a result, the need exists to qualify in vitro methods to assess the skin sensitization of compounds that may be released from medical devices, taking into account the applicability domain of known or potential skin sensitizers, including metals. To address this challenge, the predictive ability of the GARD™ assay was evaluated using a dataset of compounds known to be released from device materials. Against these data, the assay correctly predicted 19 out of 21 lipophilic and pre-/pro-hapten compounds (90.5% accuracy), with one false positive (95.2% sensitivity) and one false negative (95.2% specificity) being predicted, thus increasing the confidence in use of this in vitro assay to assess the skin sensitization potential of medical devices. Furthermore, we have also demonstrated that the GARD™ assay correctly predicts the skin sensitization response of nickel and cobalt salts (sensitizers) and a zinc salt (non-sensitizer). Overall, our data support the use of the GARD™ Medical Device Test as an AET calculated using data for in vitro methods (e.g., GMP, LLNA) that are typically used to assess skin sensitization as part of the biological safety assessment of medical devices.
Nutrients or essential elements such as calcium, sodium, potassium, magnesium, iron, zinc, and phosphorus are commonly extracted from medical devices during chemical characterization testing. A survey of recent extraction data from Medtronic implantable devices with long-term contact duration (> 30 days) or Medtronic surgical tools with limited exposure (≤ 24 days) found the following potential exposure ranges presented as μg-device: calcium (0.187 – 17.9), sodium (0.64 – 301.2), potassium (0.62 – 31), magnesium (0.013 – 7.4), zinc (0.7 – 22), iron (0.41 – 34), and phosphorus (0.06 – 17).

When device materials are subjected to extraction, extractables, including impurities, processing additives 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 with toxicological risk assessments, which evaluate potential harm from exposure to the extractables. The recently released standard ISO 10993-18:2020, which describes chemical characterization approaches, defines Analytical Evaluation Threshold (AET) and recommends its use in device extractables analysis. One of the factors contained in the AET equation is uncertainty factor (UF), which is intended to reduce the reporting limit to be inclusive of extractables that are lower responding than the level of signal that the AET is based on. This study explored considerations for curating a response factor database that is used to calculate UF values. A panel of potential medical device extractable compounds (primarily common polymer additives, a portion which are of toxicological concern) were analyzed with various instruments. Using either direct injection or headspace sampling mode, a GC/FID/MS (7890B/5977B Agilent) equipped with an internal splitter enabled simultaneous FID and MS signal acquisition. An UHPLC/UV/CAD/MS system (Dionex Ultimate 3000, Dionex Corona Ultra RS, Agilent 6540B UHD QTOF-MS) generated both UV, CAD and MS signals. Response factors of compounds were obtained by analyzing known amounts. UF necessary to be conservative with all UF-related risk in patients and no further biological testing is required. An alternative strategy of screening out these nutrients or essential elements from the list of chemicals of potential concern will also be discussed along with first-hand practical experience and challenges from regulatory agencies when the amounts of these elements are not physiologically significant.

The evaluation of biological endpoints that are relevant to the biological safety of medical devices is governed by the ISO 10993-1 standard. As part of this evaluation, a chemical characterization study may be performed followed by a toxicological risk assessment. The chemical characterization study often includes extraction with polar to nonpolar solvents for multiple days to understand the kinetic release profile and analysis by multiple instruments. The subsequent toxicological risk assessment is a systematic scientific evaluation of the potential adverse health effects resulting from human exposure to hazardous agents or situations. The purpose of this study is to focus on the evaluation of sensitization and chemical-mediated pyrogenicity endpoints on common chemicals leaching out of medical devices through a toxicological risk assessment compared to biological testing conducted in parallel. The study explored considerations for curating a response factor database that is used to calculate UF values. A panel of potential medical device extractable compounds (primarily common polymer additives, a portion which are of toxicological concern) were analyzed with various instruments. Using either direct injection or headspace sampling mode, a GC/FID/MS (7890B/5977B Agilent) equipped with an internal splitter enabled simultaneous FID and MS signal acquisition. An UHPLC/UV/CAD/MS system (Dionex Ultimate 3000, Dionex Corona Ultra RS, Agilent 6540B UHD QTOF-MS) generated both UV, CAD and MS signals. Response factors of compounds were obtained by analyzing known amounts. UF necessary to be conservative with all UF-related risk in patients and no further biological testing is required. An alternative strategy of screening out these nutrients or essential elements from the list of chemicals of potential concern will also be discussed along with first-hand practical experience and challenges from regulatory agencies when the amounts of these elements are not physiologically significant.

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When a medical device contacts a reproductive organ or releases a reproduc-
tive / developmental (R/D) toxicant, chemical characterization and tox-
ico logical risk assessment can be used to address the potential that the de-
vice might elicit R/D toxicity. However, an R/D biological test method of a
device that addresses this endpoint has not been established. To address
this gap, this study investigated if existing in silico tool/model(s) can be ap-
p lied to address the R/D endpoint for medical device chemicals by evaluating
the performance of in silico tools. A test data set of 593 reported R/D chem-
icals present in or used in the production of polymeric materials, which are
commonly used in medical devices, was mined from the European Chemicals
Agency Database. Chemicals were categorized as true positives (n=183) if
a chemical has a Globally Harmonized System R/D Classification 1 or 2 or as
true negatives (n=141) if a chemical is not classified as a R/D toxicant in the
Classification & Labeling inventory. This test dataset was then used to eval-
uate the performance of two existing publicly available in silico R/D tools,
EPA T.E.S.T. and CAESAR/VEGA. Parameters, such as the number of predicted
toxicological risk assessment can be used to address the potential that the de-
vice might elicit R/D toxicity. This analysis addresses the possibility of personalized approaches
to cancer risk assessment and prevention. For the regulatory context, we ask to
what extent can we stratify carcinogenic risk by exposure to certain products
or materials. From the personalized medicine perspective, local cancer types
most likely to be associated with device exposures such as implants are con-
sidered, along with personalized factors (i.e. genetic, environmental, lifestyle,
diet, infectious agents) that may be relevant. We conclude that the Hallmarks
of Cancer are useful when included in an evolutionary model of tumor devel-
opment. Among the KC’s, there are few measurable features beyond geno-
toxicity, thus limiting their utility. Collectively, these analyses may provide a
tool for use in cancer risk assessment for medical devices. Opportunities to
minimize regulatory burden may be found, e.g. for devices with little antici-
pated cancer risk. This approach is integrated into a current Benefit-Risk guid-
ance, to support a personalized approach to safety assessment of regulated
medical products. 

ISO 10993-18:2020 directs medical device manufacturers to assess the toxi-
cological risk assessment of medical device leachables identified by non-tar-
greated analytical screening of device extracts for unexpected and expected
extractables. However, non-targeted analytical screening can result in hundreds
of reportable extractables. Analytical screening uses non-clinically relevant
solvents of varying polarities, and the chemical data generated is typically
reported as the total quantity of each extractable released per device. Thus,
analytical screening studies require toxicologists to consider broad, often
highly conservative assumptions, regarding potential exposure and toxico-
logical risk. We investigated whether application of a toxicological screening
limit (TSL) approach could be applied to analytical screening data that re-
duces the burden of evaluating analytical screening data. Prior to conducting
a toxicological risk assessment (TRA), a TSL approach was applied to total
extractable quantities (TEQ) of identified medical device extractables that
addresses short- and long-term toxicity of a single device that contacts the
body long-term. Short (<30-day contact) and long term (>30-day contact) TSLs
were calculated by multiplying the ISO TS 21726 mutagenic Threshold
of Toxicological Concern (TTC) values of 120 or 20 µg/day, respectively, by an
assumed (conservative) number of exposure days of 1 or 30 days, respectively.
Extractables from three medical devices were categorized as either a dis-
crete chemical or a component of a mixture. Evaluations also included sum-
mation of the components in a mixture. The total quantity per device of each
discrete chemical, mixture component, and mixture were then compared to
the short and long term TSL values (i.e., 120 and 600 µg/device, respectively).
A majority of the TEQs for discrete chemicals were below the short term and
long term TSL values, while a majority of the TEQs for mixture components
and mixtures exceeded the short term or long term TSL values. We applied a
novel TSL approach that may be useful to efficiently screen identified discrete
chemicals that are reported in analytical screening studies and allow TRAs to
focus on chemicals of potential concern that may cause harm based on their
estimated exposure.
Human in vitro models to study myelin are very limited. Although myelin presents an essential function in the nervous system and impairment of this membrane is linked to many neurodegenerative diseases and neurological disorders. Thus, models and tools to study this relevant process are important to develop. We have previously developed a reproducible human iPSC-derived 3D brain model (called BrainSpheres) that contains a high percentage of wrapping myelin for in vitro model. Here, we have further developed this technology by applying different readouts to study myelination disruption. As proof of principle, we have used the BrainSpheres to assess different compounds potential to induce developmental neurotoxicity. The developing brain is susceptible to toxic insults, and there is concern that environmental chemicals contribute to widespread subclinical developmental neurotoxicity (DNT) such as autism and ADHD. Increased DNT evaluation is needed due to the lack of such information for most chemicals in common use, but in vivo studies recommended in regulatory guidelines are expensive, time-consuming and difficult to interpret. Therefore, there is an ongoing effort to develop an in vitro testing battery covering different key processes of the developing brain to possible replace current in vivo guidelines. Myelination is one of the fundamental processes in neurodevelopment that should be included in such a DNT testing strategy. Thus, there is a need to establish an in vitro myelination assay for DNT. Here we identified compounds for assay development to evaluate the relevance of our BrainSpheres model. Myelination was assessed by quantifying co-localization of myelin basic protein and neurofilament in focal images of BrainSpheres, analysis of myelin-related gene expression, and quantification of other oligodendrocyte markers. In addition, early effects on astrocytes were investigated. Results demonstrated that the positive reference compounds (cuprozine) and two newly developed myelin disruptors (BPA, MetHq and TDCPP) caused reductions in myelination in the model, while ibuprofen (negative control) did not induce these changes.

Can SARS-CoV-2 Infect Developing Human Embryos?

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SARS-CoV-2 infection is usually transmitted between humans through aero.

transisional transmission to a fetus was recently reported in a woman who developed COVID-19 in the third trimester. However, it is unknown if human embryos are susceptible to SARS-CoV-2. Our goal is to determine if SARS-CoV-2 can infect young human embryos using germ layer differentiation from human embryonic stem cells (hESCs). The entry of SARS-CoV-2 into host cells depends on the TMPRSS2 protease, which processes viral spike (S) protein and the binding of spike to its receptor, ACE2. We assessed ACE2 and TMPRSS2 expression in early stages of human embryonic development using immunofluorescence microscopy (IF) and qPCR. H9 hESCs were differentiated into the three germ layers using the Trilineage Differentiation Kit and labeled with antibodies to ACE2 and TMPRSS2 to determine when expression occurred. Differentiation was verified using specific markers for each germ layer. These included SOX17 (endoderm), nCAM (mesoderm), and PAX6 (ectoderm). Expression of ACE2 and TMPRSS2 was evaluated every other day for 5 days (endoderm and mesoderm) or 7 days (ectoderm). Based on our IF results, during ectoderm differentiation, ACE2 expression decreased on day 3, then slightly increased on day 5. ACE2 expression gradually decreased over time during mesodermal differentiation. ACE2 expression was highest in endo.

The expression of ACE2 and TMPRSS2 in hESCs was determined by qPCR and IF microscopy. IF results showed that ACE2 expression increased on day 5 and decreased on day 7 during ectodermal differentiation. During Mesodermal differentiation, ACE2 expression remained highest on both days 3 and 5. ACE2 expression peaked up on day 5 of endoderm differentiation. Co-localization of TMPRSS2 and ACE2 was observed in each germ layer. These data show that the machinery needed for SARS-CoV-2 infection is present in the surface of all three H9-derived differentiated germ layers and support the conclusion that SARS-CoV-2 could infect young human embryos in pregnant women with COVID-19. SARS-CoV-2 pseudoparticles have been generated, shown to bind to other cell types (293T-ACE2, BEAS-2B), and are currently being tested with H9. Our in vitro differentiation model combined with pseudoparticle binding will enable future testing to identify drugs that inhibit SARS-CoV-2 infection of human embryos, while simultaneously identifying drugs that may be harmful to embryonic stages of development.

Gene Network Regulations Show Different Signatures for Morpholinos and Piperidines in the ESTc

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One of the in vitro embryo toxicity screens is the cardiac embryonic stem cell test (ESTc), which uses cardiomyocyte formation as the main differentiation route. Studies are ongoing into whether a wider use of the biological domain may contribute in broadening the applicability of the test, to prioritise structurally similar compounds for its safety. The use of gene transcript biomarkers would contribute to a quantitative readout of the test. But which biomarkers could be useful for safety predictions? To answer this question, the morpholines (tridemorph - TDM; fenpropimorph - FPM) and piperidines (fenpropidin - FPD; spiroxamine - SPX) were tested. These compounds cause foetal malformations in the rat such as cleft palate. The formation of cleft palate in general is linked to interference with retinoic acid balance, neural crest cell migration, and cholesterol biosynthesis. Markers related to these pathways were tested at low (ID10) and high (ID50) concentrations after 24 hours and 7 days of exposure. All tested compounds showed stimulating effects on the cholesterol biosynthesis related marker Msmo1 after 24h exposure and TDM showed Cyp26a1 induction which codes for an enzyme that inactivates retinoic acid. A long exposure duration stimulated expression levels for pluripotency (Pou5f1), but also for the ectodermal (Nes) and endodermal (Gata4) germ layer markers. Differentiation markers were assessed for cardiomyocyte (Nkx2-5; Myh6), neural (Tubb3), and neural crest cell development (Ap2a; Mec2; Snai2; Gata4). Gene expression levels captured in gene-networks which showed clear differences in signatures between the morpholinos and piperidines, especially at low concentrations after 7 days of exposure. The different biomarker response profiles gave more mechanistic insight into the compound interferences in the tested differentiation routes which could be incorporated into the safety predictions.

Characteristics of Human iPS Cell-Derived Intestinal Epithelial Cells and Usefulness as a Model for Gastrointestinal Toxicity Evaluation

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F-hiSIEC™ (FUJIFILM) is a human iPS cell-derived small intestinal epithelial like cells. The cells show physiological characteristics similar to those of the in vivo human small intestine. For example, the most major metabolic enzyme, CYPIA4 activity was comparable to that of human primary enterocytes, and the expression of transporters such as Caco2 was captured in gene-networks which were similar to those in the human small intestinal cells. Thus, F-hiSIEC™ is a useful in vitro model for mimicking human small intestinal cells and predicting absorption of oral drugs. In order to extend the applicability of F-hiSIEC™ to gastrointestinal toxicity evaluation, several types of reference drugs were evaluated. Cytotoxic drugs; 5-fluorouracil (5-FU) is one of the chemotherapy agents for gastric, breast and colon cancers; however, gastrointestinal (GI) toxicity is a common limiting factor in clinical applications. In this study, the cell sheets of F-hiSIEC™ were treated with equivalent concentrations (1-1000 µM) of 5-FU and 5’-deoxy-5-fluorouridine (5’-DFUR) for 72 hours. The number of growing cells and barrier function were significantly decreased in the 5’-DFUR-treated group. Similar changes were also noted in the 5’-DFUR-treated group, but those were less significant. 5’-DFUR is a prodrug of 5-FU and is converted to 5-FU by a pyrimidine nucleoside phosphorylase which is richly distributed in tumor and intestine. The frequency of diarrhea caused by 5-FU and 5’-DFUR in clinical trials was 12.3% and 8.1%, respectively, and 5’-DFUR has been reported to have better therapeutic indices and safety profile than other fluorinated pyrimidines. These suggest that the in vitro system using F-hiSIEC™ can be a useful model for evaluation of GI toxic drugs and confirmation of the prodrug concept. Gamma secretase inhibitor: It has been demonstrated that F-hiSIEC™ included a wide range of secretory cells, such as goblet cells and Paneth cells as well as absorptive epithelial cells. A potent gamma secretase inhibitor, LY411575 has been developed as a therapeutic agent for Alzheimer’s disease and is known to cause diarrhea by promoting goblet cell differentiation via Notch signaling inhibition. In this study, the cell sheets of F-hiSIEC™ were treated with 0.1-10µM of LY411575. Eight days after, the goblet cell marker, MUC2 was increased and the enterocytes marker, SI was decreased in a dose-dependent manner in the LY411575-treated groups. From the results, this in vitro model using F-hiSIEC™ can evaluate effects of...
drug candidates on differentiation of intestinal epithelium. In conclusion, the present results show that the in vitro model using F-hiSIEC™ is expected as a novel GI toxicity evaluation system.

3033 A Human Embryonic Stem Cell-Based High-Throughput Platform with AI Technology to Screen for Developmental Toxicants

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Environmental factor-induced birth defects raise the risk for lifelong disabilities to those who survive and increases the economic burden to their families and society. While over 80,000 chemicals are registered for use in the United States, many of them have undergone little safety testing. Therefore, a rapid and accurate method for predicting environmental developmental toxicants in humans is strongly desired. In this study, we aim to develop a high-throughput platform with human embryonic stem cells (hESCs) and machine learning techniques to screen for environmental teratogens. Three-dimensional embryoid bodies (EBs) generated from hESCs were utilized as the model since their formation recapitulates many early embryonic processes. Thirty-five chemicals with confirmed teratogenicity at four different levels (none, minimal, moderate, and high) were employed as the standards for model training. After a 10-day exposure, the expression of 20 hallmark genes related to germ layer formation in EBs was measured for training the prediction model in 10 different machine learning algorithms. With feature selection, the Random Forest-based model showed the best accuracy (mean: 53%) and reliability (mean: 0.37) to categorize chemicals into their corresponding risk levels. To enhance and further validate the prediction accuracy, the teratogenicity of an additional 19 chemicals with limited toxicity information was assessed by this model and the results were largely consistent with previous studies. Furthermore, a complementary system using fluorescent imaging to assess chemical-elicted structural alterations in EBs was also built with a convolutional neural network (CNN) deep learning technique to help predict chemical teratogenicity. This pilot CNN model showed a high prediction accuracy (mean: 97%) and indicated potential developmental toxicity of Bisphenol A. Together, these results present a promising potential of our screening platform in identifying human developmental toxicants and understanding their etiology.

3034 New Assay Tools for Short-Term and Chronic-Dosing Cardiotoxicity Studies


Human iPSC-derived cardiomyocytes (iPSC-CM) have an important role in the development of in vitro assays to monitor cardiotoxicity with the goal of early detection, improved predictivity, and better understanding of cardiovascular pathobiology. iCell® Cardiomyocytes have been established as the preclinical in vitro model by the CIPA cross-site validation studies and peer-reviewed publications. Here we present the development of two new assay tools to assess cardiotoxic drug effects. First, the well-accepted cardiac MEA assay for measuring acute electrophysiological effects on iPSC-CM has been improved to record action potential morphologies (via "LEAP") in addition to field potentials across 96 wells. Cryopreserved iCell Cardiomyocytes were thawed into 96-well MEA plates and cultured until DIV 7. The coefficient of variation (CV) for baseline cardiac metrics (e.g., beat period) for an entire 96-well plate was reproducibly low (≤5%) across runs, from different lots of cardiomyocytes and between operators. The CIPA study (panel of 28 compounds) was repeated across four MEA plates. For the first time, compound effects on action potential morphology (e.g., triangulation or ADP90) were quantified by MEA. Next, novel assays including long-term chronic dosing studies with iPSC-CM are increasingly desirable. Moreover, use of a chemically-defined, serum-free medium to reduce the impact of compound absorption to molecules contained in serum is required. In response, we have developed a new serum-free medium (iCSFM) that has the same physiological characteristics (i.e., ionic salt concentrations, no glucose, etc.) as the standard culture medium. Cardiomyocytes cultured in iCSFM from DIV 7-14 maintained the expected purity and viability (>90% for both), as well as spontaneous beat rate (25-45 bpm) over time. Repeat dosing with cardiotoxic molecules (e.g., doxorubicin and pentamidine) in iCSFM this medium showed disturbances in electrophysiology, contractility, and cell viability that do not appear with short-term measurements. This study highlights the new assay tools we have developed for the expansion and advancement of toxicity and safety studies, as well as the value of both short- and long-term assessment with iPSC-derived cardiomyocytes.

3035 Evaluation of the hPSC Scorecard Assay in a Human Embryonic Stem Cell Test for Developmental Toxicity Screening

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The US EPA employs high-throughput screening approaches to identify environmental chemicals that can pose a risk to human health. Key provisions in the Frank R. Lautenberg Chemical Safety for the 21st Century Act promotes the use of non-animal, new approach methods to identify chemical risks to susceptible populations including pregnant women. Most of the current assays within the US EPA’s ToxCast and Tox21 portfolio are not designed to evaluate cellular processes associated with human development, therefore cell-based models that recapitulate signaling pathways for defined endpoints in early embryonic patterning are needed to identify potential hazards during pregnancy. The objective of this study was to adapt human induced pluripotent stem cells (hiPSCs) to a 96-well embryoid body (EB) stem cell test to identify chemicals that perturb early germ layer differentiation. EB formation, maintenance, and treatment was optimized for seeding density, plate type, and media changes using automated liquid handling. The commercial TaqMan hPSC Scorecard Assay gene-signature array was used to conduct temporal analysis of spontaneous differentiation. Pronounced EB differentiation was noted as early as day six, with maximum germ layer expression plateauing at day ten. Solvent tolerance and concentration-range finding experiments were used to define a reference set of eight chemicals (all-trans retinoic acid, aspirin, caffeine, diethylenetetraacetate, folate acid, saccharin, thalidomide and valproic acid) ranging the spectrum of FDA pregnancy risk categories (A-X) at known teratogenic and non-teratogenic doses. Disruption of germ lineage commitment and progression was determined after six days of chemical exposure by calculating the difference in Z-test scores (ΔZ-test) relative to pre-aggregated cells. Solvent treated controls displayed successful EB differentiation with ΔZ-test values for ectoderm (18.8 ± 5.1), mesoderm (4.5 ± 1.8) and endoderm (11.1 ± 2.2). Only all-trans retinoic acid (0.01µM) treatment provided a difference in EB differentiation with increased expression in endoderm genes (21.7 ± 4.1). Further investigation is warranted to determine if exposure frequency, duration, and endpoint analysis are suitable for evaluating hiPSC EB differentiation using the TaqMan hPSC Scorecard Assay gene panel. The views are those of the author and do not necessarily reflect the views or policies of the US EPA.

3036 Comparison of Immunoconditioning Regimens for Cell Therapy Toxicology Studies Using the NCG or NSG Mouse Models


Toxicology studies for cell therapies require animal models that enable non-invasive, high-throughput screening of drug candidates on differentiation of intestinal epithelium. In conclusion, the present results show that the in vitro model using F-hiSIEC™ is expected as a novel GI toxicity evaluation system.
In the US, racial disparities in breast cancer outcomes between women of African and European ancestry have been characterized for decades, yet the biological basis for these disparities remains elusive. Mounting evidence points to an important role for stem cells in breast cancer, and studies have linked normal breast stem cell number with genetic and environmental risk factors. African American communities are disproportionately exposed to environmental toxicants which may contribute to disparities observed in breast cancer. Of these, bisphenol-A (BPA) and its analogues are of interest due to their ability to alter mammary gland morphogenesis and stemness in vivo and in vitro. This study aims to characterize the differences in normal breast stem cell biology between women of African and European ancestry and the effects of bisphenols on normal stem cells. Cryopreserved punch biopsy samples from healthy, nulliparous women were obtained from the Susan G. Komen normal tissue bank. A European American (AA) and 4 European American (EA) samples were established in conditional reprogramming culture conditions, which induce expansion and dedifferentiation of primary cells. We assessed the effect of bisphenol-A, S, and F in 2D and 3D culture at human relevant doses (25µM-10M). Low doses of BPA and BPS (100nM, 1µM, 10M) increase mammosphere formation up to 30%. Samples were profiled using single-cell RNA sequencing and unbiased clustering revealed that all samples retained luminal and myoepithelial cell populations, with proportions varying by individual. AA samples were enriched for an embryonic stem cell gene expression signature, particularly in the myoepithelial population. Differential gene expression between AA and EA samples revealed 6867 differentially expressed genes (DEGs) in the luminal subtype and 5385 DEGs in the myoepithelial subtype. Of particular interest is a driver of pluripotency, and CRYPB2 (log2FC=-4.5), associated with AA ethnicity and survival in multiple cancer types. These findings provide insight into the relationship between stem cells, cancer, and the environment and how they may impact breast cancer disparities.

Arsenic is a ubiquitous toxic metaboloid that affects over 150 million people through the ingestion of contaminated food and water. Arsenic exposure during pregnancy and embryonic development can impair cellular differentiation, leading to altered neuronal and muscle development. The goal of our study was to investigate the mechanisms by which chronic arsenic exposure disrupted cell differentiation. To assess our objectives, mouse embryonic stem cells (ESCs) were chronically exposed to 0.1 µM (7.5 ppb) arsenic for 32 weeks. Cells were differentiated every 4 weeks and were collected at day 5 as embryoid bodies (EBs) for immunohistochemistry analysis, and at day 9 as differentiated cells for RNA and protein analysis. RNAseq followed by PCA analysis indicated that arsenic-exposed ESCs express different genes than their control counterparts and these differences increase over time. Moreover, pathway analysis indicated that the Hippo pathway might be disrupted following chronic arsenic exposure. Specifically, transcript expression of Hippo pathway key components Lats2, Tead3 and Tead4 was significantly upregulated by 1.7, 2.1- and 2.5-fold change in arsenic treated samples at week 24, respectively. The Hippo pathway is a conserved signaling pathway involved in embryonic development that regulates cellular differentiation and stem cell maintenance. Specifically, previous studies have reported that the Hippo pathway’s main effector Yap is upregulated in pluripotent stem cells; interestingly, preliminary data show that protein expression of active Yap1 begins to increase at week 0 and is significantly upregulated by 4.6-fold in arsenic treated samples at week 8. Cadherin/catenin complex is involved in the initiation and signaling of the Hippo pathway and, interestingly, arsenic exposure significantly increased N-cadherin protein expression between 1.3- and 1.8-fold beginning at week 16 throughout the rest of the exposure as N-cadherin positive cells grouped at the perimeter of arsenic-exposed EBs. Conversely, no significant differences were reported for E-cadherin protein levels in control and arsenic-treated EBs. Taken together, these results suggest that chronic exposure to an environmentally relevant arsenic concentration might impair cellular differentiation of ESCs through the disruption of the Hippo pathway signaling.
The core function of hematopoietic stem and progenitor cells (HSPCs) is the production and maintenance of the proper balance of all lineages of blood and immune cells throughout an organism’s life-span. The mechanisms that modulate HSPC lineage specification are not fully understood. The aryl hydrocarbon receptor (AhR), an environment sensing transcription factor, has been implicated as a regulator of hematopoiesis. For instance, AhR ligands modulate the frequency of mature hematopoietic cells in the bone marrow and periphery, and mice lacking AhR (AhR-KO) have an abnormal frequency of HSPCs. We hypothesize that AhR regulates HSPC homeostasis and directs differentiation towards specific lineage-biased progenitors. Our current study reveals that AhR-KO mice have an increased proportion of myeloid-biased HSCs and myeloid-biased progenitors are driven by AhR signaling that is intrinsic to the hematopoietic compartment. Using hematopoietic specific conditional AhR knockout mice (AhRvav1), we determined that increased frequency of myeloid-biased HSCs and myeloid-biased progenitors are driven by AhR signaling that is intrinsic to the hematopoietic compartment. AhR-KO mice do not express AhR at any point in their lifespan, thus changes to HSPCs may due to chronic lack of AhR. Utilizing novel inducible AhR knockout mice (iAhR-KO), it was revealed that acute deletion of AhR doubled the number of MPP3 cells and also altered the composition of downstream lineage-committed progenitors, including increased frequency of primitive granulocyte/macrophage committed progenitors. To further test whether myeloid-biasing was an effect of the acute attenuation of AhR, we used the AhR antagonist CH-223191. Consistent with inducible AhR antagonism promoted myeloid-biased differentiation. These findings demonstrate that the AhR plays a pivotal role in regulating steady-state hematopoiesis, influencing HSPC homeostasis and lineage potential. Additionally, these findings provide further insight into how dysregulation of AhR signaling through environmental exposure can potentially affect the ability of HSPCs to properly respond to stressors.

Oxidant-Induced Epithelial Alarmin Pathway Mediates Lung Function Decline after Ultrafine Carbon Black and Ozone Inhalation Co-exposure

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Air pollution is a complex mixture of particulate matter and gaseous substances. Inhalation co-exposures represent more realistic exposure scenarios, however, there are only a limited number of studies detailing biological impacts of inhalation co-exposure. We hypothesized that co-exposure induce significantly higher toxicity through unique biological pathways. We exposed C57BL/6J mice to air, 10 mg/m³ ultrafine carbon black (CB) and 2 ppm ozone separately or together at for 3 hours (18 µg particle deposited dose). Lung function was assessed by Flexivent system. Bronchoalveolar lavage (BAL) was performed and the lung tissue collected 24-72 hours post exposure. Aerosols of similar number and mass-based distributions were generated and extensively characterized. Significant increases in acellular and cellular oxidants were observed in co-exposure scenario using electron paramagnetic resonance (EPR) measurements and flow cytometry. In addition to a synergistic increase in lung inflammatory cell influx and lung injury, a significantly greater gene and protein expression of thymic stromal lymphopoietin (TSLP) and a number of inflammation related markers (IL-6, IL-13, KC, TNF-α, IL-1β) was noted in co-exposure group compared with individual exposure groups. Treatment with a SOD/catalase mimetic EUK-134, and TSLP neutralizing antibody resulted in a significant attenuation (~2 folds) of inflammatory cytokine gene and protein expression. These interventions also resulted in improved lung function measures such as respiratory resistance and forced expiratory measurements. In conclusion, utilizing a more realistic particle and gas co-exposure inhalation, we demonstrate that oxidant induced epithelial TSLP production plays a central role in co-exposure induced lung inflammation and decline in lung function. Funding: SH (ES031253), SH (U54GM104942-03), TRN ES (015022).

Suppression of Ozone-Induced Alveolar Macrophage Activation and Increased Mitochondrial Respiratory Function in Mice by Valproic Acid


Acute exposure to inhaled ozone causes tissue injury, oxidative stress, inflammation, and impaired pulmonary mechanics. This is associated with an accumulation of inflammatory macrophages in the lung which contribute to pathophysiologically responses to ozone. In the present studies we assessed the effects of valproic acid (VPA), a histone deacetylase inhibitor with antioxidant and anti-inflammatory activity, on ozone-induced activation of alveolar macrophages (AM) and oxidative stress. C57Bl/6J mice were exposed to air or ozone (0.8 ppm, 3 h) in whole body chambers. This was followed 30 min and 24 h later by i.p. VPA (300 mg/kg). Mice were euthanized 48 h later and bronchoalveolar lavage fluid (BAL), AM and tissue collected. Flow cytometric analysis showed that AM accumulating in the lung following ozone exposure were phenotypically pro-inflammatory as measured by high expression levels of Ly6C (Ly6C+) AM. AM also expressed TNFα and ADP-ribosylation factor-like protein (ARL)-11, demonstrating that they were activated. Ozone exposure also resulted in increased mitochondrial respiration in AM measured using an Agilent Seahorse Analyzer. Treatment of mice with VPA significantly reduced the number of activated proinflammatory macrophages accumulating in the lung in response to ozone, as evidenced by decreases in numbers ofLy6Chigh cells and reduced expression of TNFα and ARL-11. This was associated with a reduction in AM mitochondrial respiration. VPA also blocked ozone-induced oxidative stress, as measured by expression of heme oxygenase (HO-1) and 4-hydroxynonenal (HNE) modified proteins in AM, and by decreases in the rate of NADPH-dependent H2O2 production in the lungs. Mice exposed to ozone exhibited aberrant pulmonary function, including increases in central resistance and tissue elastance; these changes were dampened by VPA. Taken together, these data demonstrate that VPA reduces ozone-induced inflammation, oxidative stress, and mechanical dysfunction. These findings may be useful in the development of therapeutics to treat oxidant-induced lung injury. Supported by NIH Grants ES005022, ES004738 and AR055073.
Increased levels of ambient ozone, one of the six criteria air pollutants, result in the respiratory tract injury and worsening of ongoing lung diseases. However, the effect of ozone exposure on the respiratory tract undergoing active lung development and simultaneously experiencing mucoinflammatory lung diseases such as cystic fibrosis (CF) remains unclear. To address these questions, we exposed sodium channel non-voltage-gated 1, beta subunit overexpressing transgenic mice (Scnn1b-Tg), a mouse model of CF-like lung disease, and littermate wild type (WT) mice to ozone from postnatal day (PND) 3-20 and examined the lung phenotypes at PND21. Total cell counts, differential cell counts, cytokine estimation, and microbiological analysis were performed on bronchoalveolar lavage fluid. Formalin-fixed lungs were analyzed for histopathological assessment and whole lung digest was processed for gene expression analyses. As compared to filtered air (FA)-exposed WT mice, the ozone-exposed WT mice exhibited marked alveolar space enlargement, in addition to significant eosinophil infiltration, type-2 inflammation, and mucous cell metaplasia. Ozone-exposed Scnn1b-Tg mice also exhibited significantly increased alveolar space enlargement, which was also accompanied by exaggerated granulocytic infiltration, type-2 inflammation, and greater degree of mucocoobstruction. The alveolar space enlargement in ozone-exposed WT, and ozone-exposed Scnn1b-Tg mice was accompanied by elevated levels of MMP12 protein in macrophages and Mmp12 mRNA in the lung homogenates. Finally, while bacterial burden was largely resolved by PND21 in FA-exposed Scnn1b-Tg+ mice, ozone-exposed Scnn1b-Tg+ mice exhibited compromised bacterial clearance which was also associated with increased levels of IL-10, an immunosuppressive cytokine, and marked mucocoobstruction. Taken together, our data show that ozone does not result in alveolar space remodeling during active phases of lung development and markedly exaggerates the developmental history of an ongoing mucoinflammatory pediatric onset lung disease by worsening disease outcomes including bacterial infections, granulocytic inflammation, mucocoobstruction, and alveolar space enlargement.

Ozone Exposure Differentially Affects Inflammatory Response in Primary Human Nasal Epithelial Cells from Males and Females

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Ground-level ozone inhalation has been linked to numerous health complications including airway and lung inflammation, with some studies in mouse models indicating sex-based differences. Ozone reacts with cholesterol in the lung, leading to the formation of highly electrophilic oxysterols. Oxysterols can then interact with nucleophilic centers of proteins, altering their expression and causing downstream transcriptional or functional alterations. As there are baseline respiratory immune gene expression and cholesterol profile differences between males and females, we aimed to determine whether ozone-derived oxysterol formation and their activation of inflammatory pathways may be sex-dependent. To address this gap in understanding, we completed a study of controlled ozone exposure on primary human nasal epithelial cells (HNECs) derived from healthy human males and females. HNECs were acquired by nasal scrape biopsy, then grown and differentiated in culture to air-liquid interface (ALI). At ALI, cells were exposed to either filtered air or 0.4 ppm ozone for 4 hours. Apical washes, basolateral supernatants, and cell lysates were then collected 1 hour post-exposure and analyzed for immunomediator release via ELISA, gene expression profiles via qPCR, and HPLC-MS for oxysterol formation. Expression of SERPINB3 was significantly increased following ozone exposure, but no sex-based trend was identified. In apical wash samples, Eotaxin and IL-16 release increased with ozone exposure in male-derived cells, but not in female-derived cells. The same trend was not seen in basolateral supernatants. Contrastingly, analysis of basolateral supernatant samples revealed urotheloglobin release increased in females but not males after ozone exposure. Additionally, the concentration of oxysterol molecule sec-dihydroxycholesterol B was significantly increased in apical wash samples in females but not in males. These results indicate that ozone exposure may induce sex-specific respiratory immune responses which may be due to sex-specific formation of ozone-induced oxysterols.

Modelling the Pulmonary Transcriptome in a Dose- and Substrate-Dependent Manner: Carbon Black and Ozone Co-exposure

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While investigating single inhaled toxicants may be useful, the additive effects of multiple toxicants interacting within the body can only be modelled through co-exposure. We evaluate how inhalation of carbon black (CB) and ground-level ozone (O3) alters the lung transcriptome through single or multiple-dose exposures, and if co-exposure can incite unique genome-wide changes. C57BL/6 mice were exposed to CB (10 mg/m3) and/or O3 (2 ppm) for 3 hours (either a single exposure or four independent exposures). RNA was isolated from lungs and mRNA sequencing performed using the Illumina HiSeq. Lung histology and immunohistochemistry were performed. Electron transport chain (ETC) activities and electron flow were assessed. Co-exposure groups revealed significantly greater total number of cells, neutrophils and macrophages in the lung lavage compared with other groups. At Day 1 and 4, co-exposure revealed the most differentially expressed number of genes (2234 and 4044, respectively) of these genes, 1188 (Day 1) and 2061 (Day 4) were uniquely differentially expressed, with 33 mitochondrial ETC mRNA transcripts significantly impacted at Day 4. Both O3 and co-exposure treatment significantly reduced ETC maximal activity for complexes I (-39.3% and -36.2%, respectively) and IV (-55.1% and -57.1%, respectively). Only co-exposure reduced complex V activity (-35.7%). CB and O3 co-exposure can cause unique transcriptomic changes in the lungs, decreasing electron flow and impairing mitochondrial bienergetics. Funding: SH (ES012723), SH (U54GM110494-03), TRN (ES 015022), JMH (HL-128485), AK (AHA-20PRE35080170), Community Foundation for the Ohio Valley Whiskey Trust.

Absence of ALX/FPR2 Reduces Pulmonary CCL2 during the O3-Induced Pulmonary Inflammatory Response

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Ozone (O3) is a criteria air pollutant that contributes to the morbidity and mortality of cardiopulmonary diseases. O3 is known to induce pulmonary inflammation and injury as characterized by immune cell influx into the airspace, pro-inflammatory cytokine/chemokine production, and oxidative stress. Following the induction of inflammation, the resolution phase of inflammation is activated to resolve inflammatory responses, return the lung to homeostasis, and prevent chronic inflammation. The resolution of inflammation is governed by the production of specialized pro-resolving mediators such as D-series resolvins (RvDs). We recently published that RvD1 receptor, formsyl peptide receptor 2 (ALX/FPR2), are decreased in the lung after O3 exposure. ALX/FPR2 is a G protein coupled receptor, mainly expressed on phagocytic leukocytes, that when activated can inhibit the production of NF-κB and upregulate expression of anti-inflammatory genes. We hypothesized that ALX/FPR2 contributes to resolving the pulmonary inflammatory response to O3. To test this hypothesis, we exposed male ALX/FPR2 knockout (KO; on a C57BL/6J background) and FPR2 WT littermate control male mice to 1 ppm O3 for 3 hours. 24 hours later, during peak inflammation, markers of pulmonary inflammation, injury, and oxidative stress were assessed. WT and KO mice had no differences in markers of lung injury, immune cell infiltration into the airspace, or pulmonary expression of pro-inflammatory cytokine/chemokines, or antioxidant response genes. Notably, following O3 gene expression of the monocyte chemoattractant CCL2 was significantly decreased in the lung tissue of ALX/FPR2 KO mice. These findings suggest that ALX/FPR2 contributes to monocyte chemotaxis to the lung following O3. Future investigations will elucidate the role of ALX/FPR2 during later time points (during heightened resolution) after O3 exposure, and will evaluate the impact of ALX/FPR2 on monocyte chemotaxis.
Alterations to hepatic function, including transporter expression, during progressive nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) are well documented. Recent evidence suggests that renal function, including glomerular filtration, is also perturbed during these diseases. To avoid adverse drug reactions, an accurate phenotype of renal xenobiotic transporters in NAFLD and NASH patients is critical. To evaluate this, formalin-fixed and paraffin-embedded kidney needle biopsies were acquired from patients (n=5-6/group) identified with paired diagnoses of NAFLD, NASH, or healthy liver. Protein was extracted and then digested with trypsin to generate unique surrogate peptides for 34 clinically relevant transporters; these surrogate peptides were then quantified against pure standards by LC-MS/MS. Trend analysis demonstrated transporter protein abundance decreases during disease progression from healthy to NAFLD of: BCRP (0.15 to 0.06 pmol/mg protein), OCT2 (157 to 17 pmol/mg protein), MRPs (0.42 to 0.24 pmol/mg protein), ENT1 (28.5 to 10.8 pmol/mg protein), and CNT3 (0.19 to 0.09 pmol/mg protein). Only ASBT, a renal bile acid transporter, increased from 1.7 to 5.3 pmol/mg protein during disease progression. OAT4 expression decreased from healthy protein abundance of 1.72 to 0.78 in NAFLD and 0.47 pmol/mg protein in NASH patients. Interestingly, abundance of the alpha subunit of Na+/K+-ATPase decreased from 14.8 pmol/mg protein in healthy patients to 5.3 and 3.3 in NAFLD and NASH patients, respectively. These findings are the first to quantify alterations to renal xenobiotic transporters in patients with progressive stages of NAFLD. Notably, expression of transporters involved in renal secretion of organic anions (OAT4 and Na+/K+-ATPase) are considerably reduced, suggesting a novel mechanism by which these patients may be at greater risk of adverse drug reactions. As such, appropriate models recapitulating these changes are warranted to study their putative effects on the toxicokinetics of clinically relevant substrates.
Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy) propanoic acid (GenX/Hexafloc-98-9) is a novel fluoroalkylated perfluorooctanoic acid (PFAS) that was introduced as the replacement for perfluorooctanoic acid (PFOA). GenX pollution of the Cape Fear watershed in North Carolina for over a decade resulted in contaminated ground and finished drinking water. We recently showed GenX downregulates the activity of ABCB1 (P-glycoprotein/ PgP) and ABCG2 (Breast cancer resistance protein/BCRP) using in vivo, in vitro, and ex vivo assays. Maintenance of barrier tissues is dependent on a well-coordinated system of tight junction proteins and active transport proteins to protect and preserve sensitive cell populations. In addition, efflux of many therapeutic drugs is regulated by ABCB1. One such drug is the calcium channel blocker verapamil. To determine whether in vivo exposure to GenX alters disposition of a representative ABCB1 substrate, we co-administered radiolabeled [3H]-verapamil (0.1 µCi/mL) with GenX (0.125 nmol/mL, approx. 30 nmol/kg) for 20 minutes via an indwelling carotid artery cannula in male Sprague-Dawley rats and measured [3H]-verapamil in brain, kidney and liver. Brain perfusion studies found statistically significant increases in [3H]-radioactivity in brain after a co-perfusion of [3H]-verapamil and GenX. Whole-trunk infusion of [3H]-verapamil and GenX found statistically significant increases in [3H]-radioactivity disposition in liver and kidney. These data are consistent with our earlier studies that show that GenX inhibits ABCB1 activity, reduces barrier function, and allows more substrates to enter the brain (CNS). These results demonstrate that GenX exposure has the potential to alter in vivo disposition and pharmacokinetics of clinically used drugs that are ABCB1 substrates, with both public health and adverse drug reaction implications. This research was supported by the NIH Intramural Research Program (ZIA ES013348).

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Fatty esters are common compounds requiring structure activity relationship (SAR)-based safety assessments, and a large portion of those esters involve a component derived from 2-ethylhexanoic acid (EHA) or 2-ethylhexanol (EH). EHA is a branched-chain fatty acid with the potential to display developmental or reproductive toxicity (DART). This work uses human liver S9, skin S9 and plasma to measure the in vitro hydrolysis of a series of fatty esters of EHA or EH with alkyl chains ranging from C1 to C11. The in vitro intrinsic clearance results show that metabolism rates vary between test matrices, carbon chain length, and functional group (EHA or EH). In the liver, intrinsic clearance rates were generally higher for esters of EHA than for esters of EH. Esters of EH had an increasing rate of liver metabolism from C1 to C5, which then decreased from C9-C11. For esters of EH, the rate of liver metabolism was comparable from C2-C5 and then decreased from C9-C11. In vitro intrinsic clearance rates for liver were 300- to 2500-fold higher than skin. The rates of skin metabolism for esters of EHA was unimodal and peak activity occurred for the C4 variant, whereas rates of skin metabolism for esters of EH decreased with increasing acyl chain length. In general, the plasma had low to no enzymatic activity towards the esters of EH. In vitro to in vivo extrapolation was conducted on all data to determine the in vivo relevance of these metabolism-based SARs. When assuming unrestricted metabolism and using the well stirred hepatic model, the in vivo metabolism for all the chemicals would be hepatic blood flow limited since the hepatic blood flow was much slower than the hepatic intrinsic clearance rate. In silico estimates of skin permeability and the in vitro rates of skin metabolism were used to estimate dermal bioavailability. These esters are expected to be systemically bioavailable since the rate of permeability is faster than the rate of skin metabolism. When scaling the in vitro metabolism data for plasma, all esters are expected to experience minimum metabolism by plasma as indicated by predicted in vivo elimination rates of 0.02/hr or less. Overall, these data demonstrate that branching in the 2-position of alkyl esters of EHA and EH impacts the rate of ester bond hydrolysis.

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Chlorinated derivatives of Bisphenol A (ClxBPA) are formed from Bisphenol A during chlorination of water, including tetrachlorobisphenol A (Cl4BPA). Cl4BPA was found in tap water as well as in human biological matrices like urine, serum of pregnant women and adipose tissue. This constitutes a potential risk to human health. The aim of this study is to determine toxicokinetic in Sprague-Dawley rats for tetrachlorobisphenol A in order to better evaluate the distribution process in rats. We exposed 20 male Sprague-Dawley rats (280g) intravenously to Cl4BPA at two different doses: 0.4 mg/kg and 4 mg/kg (10 rats per dose). Blood samples were taken from the lateral vein of the tail at 7 different times: 0.25, 0.5, 1, 2, 4, 8 and 24 h. After euthanasia, we have collected different tissues such as liver, brain, muscles and adipose tissue at 4 and 24 h, and harvested urine and feces at these times. Cl4BPA concentrations in plasma and tissues were determined by using a validated LC-MS/MS assay. The plasma kinetic curve at the lowest dose can be described by a bicompartiment kinetic model. Kinetics at the highest dose is also biexponential but concentrations are lower than the factor 10 expected compared to lowest dose. This difference could be explained by a possible saturation in the distribution process (plasma binding or transport). Moreover, excretion in feces was much greater compared to urine. Indeed, at 4h for 0.4 mg/kg, feces concentrations were 28.5 times greater than in urine, and 575 times greater at 24h. Finally, we noticed that tissue: plasma concentration ratios for all tissues are lower than 0.2, except for the liver which is greater than 1 at 4 and 24 h postdosing. These suggests that there is a weak distribution in these tissues (brain, muscles and fat) and possibly hepatic active transport explaining also the observed fecal excretion. Further investigations are required to understand the kinetic mechanisms of Cl4BPA in order to develop a PBPK model for exposure assessment. In addition, the toxicokinetic of the other ClxBPA are also currently under investigation.
3057 Variation in Methylmercury Metabolism and Elimination in Humans: PBPK Modeling Highlights Roles for Gut Biotransformation, Skeletal Muscle, and Hair

The biological half-life \( (t_{1/2}) \) of methylmercury (MeHg) shows considerable individual variability \( (t_{1/2} < 30 \text{ to } > 120 \text{ days}) \), highlighting the importance of understanding the underlying mechanisms controlling MeHg metabolism and elimination. Building on prior physiologically based pharmacokinetic (PBPK) model, we elucidate parameters that have the greatest influence on variability of MeHg \( t_{1/2} \) in the human body. Employing a unique dataset of parameters for mean organ volumes and blood flow rates appropriate for man and woman (25-35yrs) and child (4yrs), we demonstrate model fitness by simulating data from our prior controlled study of MeHg elimination in people. Model predictions agreed with MeHg \( t_{1/2} \) of 46.9, 38.9 and 31.5 days and steady state blood MeHg of 2.6, 2.6 and 2.3 \( \mu g/L \) in man, woman and child, respectively, subsequent to a dose of 0.7ug/kg body weight. The major routes of elimination were biotransformation of MeHg to inorganic Hg (Hg) in the gut lumen (73% in adults, 61% in child) and loss of MeHg via excretion within growing hair (13% in adults, 24% in child). Local and global sensitivity analyses of model parameters revealed that variation in MeHg biotransformation rate in the gut lumen, and rates of transport between gut lumen and gut tissue, have the greatest influence on MeHg \( t_{1/2} \). Volume and partition coefficients for skeletal muscle and gut tissue also showed significant sensitivity affecting model output of MeHg \( t_{1/2} \). Our results emphasize the role of gut microbiota in MeHg biotransformation, transport kinetics at the level of the gut and skeletal muscle mass and fitness as moderators of MeHg kinetics in the human body. Supported by National Institute of Environmental Health Sciences R21ES024859, RO1ES025721 and P30ES001247.

3058 Was It Really Necessary? Generation of Additional Toxicokinetic Data Designed to Identify a Point of Inflection
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Aldophorpen (AF) is an insecticide that acts as a TRPV channel modulator in chordotonal organs of target insects and has been assessed for a wide range of toxicity endpoints including chronic toxicity and carcinogenicity studies and toxicokinetic (TK) studies in rats and mice. Previous studies in the rat revealed nonlinear TK for AF at doses > 15 mg/kg bw/d (dose disproportionate increase of parent compound in plasma). In rats, the available data support that onset of nonlinear plasma PK is likely due to the saturation of N-oxidation of AF to metabolite M4401017. This metabolic saturation results in dose disproportionate increases in parent AF in the plasma. This study was designed to evaluate the TK of AF in rats at doses below and above those previously demonstrated to result in non-linear kinetics (3-15 mg/kg bw/d). In this study, linear kinetics were observed at dose levels of 0.1, 0.5 and 2.5 mg/kg bw/d in both males and females after 21 and 28 days of dietary dosing. However, likely biologically relevant dose-disproportionate increases were observed at doses of 12.5 mg/kg bw/d and greater. Based on the results of this study, the kinetically derived maximum dose (KMD), is estimated to be between 2.5 and 12.5 mg/kg bw/d. Although this study provides additional evidence of the range of doses at which kinetics begin to shift from linear to non-linear identified in prior studies, a precise point of inflection could not be defined and minimal refinements were made to the range of the KMD. The findings of this study demonstrate the impossibility to define a definitive point of inflection, where kinetics change from linear to non-linear, a finding that is supported by physiological mechanisms of enzyme kinetics. The range of the KMD for AF, 2.5 - 12.5 mg/kg bw/d, further supports the non-relevance of adverse biological effects seen in the AF toxicological database at dose levels above 12.5 mg/kg bw/d. These data demonstrate that consideration of TK is critical for improving dose selection in toxicity studies as well as to enhance human relevance of interpretation of animal toxicity studies and the technical difficulty in obtaining a defined point of inflection from in vivo TK data.

3059 Expression Profiling of Xenobiotic Processing Genes in Human Placentas across Gestation
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The placenta protects the fetus and transports nutrients while allowing for waste excretion. As a metabolically active tissue, the placenta is comprised of enzymes and transporters that can impact xenobiotic biotransformation and in turn, development of the fetus. The processing of drugs and other chemicals by the placenta is regulated by uptake and efflux transporters and drug-metabolizing enzymes. In this study, we sought to evaluate the mRNA expression of 250 xenobiotic-metabolizing enzymes and transport genes and their transcriptional regulators in healthy human placentas from mid-term to term pregnancy. Using existing RNA-sequencing data (GSE124282), differences in mRNA expression in human primary cytrophoblasts were evaluated at mid-term (18-22 weeks, \( N = 4 \)) and term (38-40 weeks, \( N = 4 \)). Mean FPKM values were calculated for each gene and used to assess the overall abundance (FPKM > 1) at mid-term and term gestation. Identification of differentially expressed genes was performed using EdgR and technical variations were controlled using RUVSeq (FDR < 0.05). Of the 250 xenobiotic processing genes evaluated, 59% and 93% were expressed in trophoblasts at mid-term and term, respectively. The most highly expressed gene families included HSD (hormone pathways), ALDH (phase I metabolism), GST (phase II metabolism), SLC (uptake/bidirectional transporters), and ABC (efflux transporters) at both gestational time points. As expected, time-dependent expression was observed for hormone-related genes including those expressed at midterm (CGA, HSDB32, HSDB791), CYP1A1 and 19A1 and term (CRH). The most highly expressed xenobiotic processing genes enriched at mid-term included the phase I enzyme EPHX1 (3-fold) and the uptake carrier SLC0281 (18-fold) (mid-term/term). By late pregnancy, higher mRNA expression of phase I enzymes CES1 (29-fold), ALDH1A2 (3-fold), and ALDH2 (3-fold), as well as uptake transporters SLC22A1 (13-fold), SLC22A1 (2-fold), SLC04A1 (2-fold) and SLC11A1 (4-fold) term/mid-term was observed. In conclusion, we have identified unique placental gene expression signatures at different developmental stages, and an overall increase in cumulative mRNA counts over time, suggesting an increasing need for detoxifying chemicals following fetal exposure to xenobiotics. Supported by NIH ES020721, ES001748, ES050922, TR00317, and ES052975.

3060 Assessing the Relationship between Neighborhood Socioeconomic Status and Toxic Chemical Releases in Upstate New York
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It has previously been demonstrated that certain demographic groups – particularly minorities and low-income individuals – live disproportionately closer to polluting facilities. Therefore, we investigated the chemical releases from Toxic Release Inventory (TRI) facilities in four counties in Upstate New York (Albany, Erie, Monroe, and Onondaga Counties). The chemicals released were weighted based upon their potential toxicity to determine if any demographic groups were being more heavily exposed to hazardous chemicals. Using hierarchical clustering, we created seven distinct clusters from nine population demographics representing neighborhood-based socioeconomic status. We then geocoded the TRI polluting facilities into the residential clusters and calculated the quantity, in pounds, of chemicals released. Facility Scores, which are determined based upon the toxicity of the chemicals the facilities are releasing, from EPA’s Risk-Screening Environmental Indicators (RSEI) Model were obtained and the top five facilities with the highest Facility Scores per cluster were examined in more detail. Three clusters, the Minority Working Class, Working Class, and Wealthy Working Class clusters, had Facility Scores that were weighted based on population demographics more than the Facility Scores in other clusters. Looking at the demographics of these clusters, we found that those working in non-managerial positions were the most affected group in our study area. When we assessed the quantity of chemicals released in these clusters, the Minority Working Class and Working Class clusters had the two highest quantities of chemicals released and the Wealthy Working Class cluster had one of the lowest quantities of chemicals released, suggesting that using the quantity of chemicals released alone may be misleading in terms of potential toxicity and exposure. This also suggests that communities that have TRI facilities that report minimal quantities of chemical releases may be at the same level of risk of pollutant exposure as the communities that have TRI facilities reporting large quantities of chemical releases.
3061 Clinical Management of Poisoned Patients: An Educational Intervention for Medicine Students of the University of Concepción, Chile

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On one hand, Chilean undergraduate medicine students are vaguely familiar with clinical toxicology since Chilean universities do not include toxicology courses as mandatory so far. On the other hand, the local poison control center has been reporting increases related to phone calls associated with poisonings. Specifically, 57% of the phone calls made from 2006 to 2015 come from health care settings. This is a remarkable situation that must be considered specially if undergraduate medicine students do not have basic knowledge in regards with toxicology. The objective of this work was to provide basic knowledge in the field of clinical toxicology to undergraduate medicine students through electronic learning material such as videos and a pocket guide for the treatment of poisoned patients. As to methods, an educational intervention for 6th and 7th grade medicine students through electronic learning material such as videos and a pocket guide for the treatment of poisoned patients. As to methods, an educational intervention for 6th and 7th grade medicine students at the University of Concepcion, Chile was designed and executed. This intervention was based on a 20-hour e-learning clinical toxicology course. Students improvement was determined by a guided questionnaire applied prior and after taking the course. General results showed that from the 103 participants, correct answers to questions from the questionnaire increased from 53% to 75% prior and after the educational intervention respectively. Similarly, prior to the intervention 9% of participants declared to have medium-to-high level of knowledge about toxicology. This percentage increased up to 60% after the intervention. Also, 100% of participants passed the final exam associated with the educational intervention with an average grade of 6.9 out of 7.0. Additionally, 79% of participants indicated that toxicology courses should be taught to undergraduate medical learners. Finally, the educational intervention, including the e-learning course, along with the learning material contributed to improve participating medicine students’ skills and knowledge related to clinical toxicology, especially on the management of poisoned patients.

3062 Diversity Initiatives in Undergraduate Research and Education: Our Journey towards Success in Meeting the Goals of 2nd CDI-UDP 2020 Cycle

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The goals of the mentoring group 2 of CDI-UDP of SOT 2020 cycle include the following: a) To promote awareness among undergraduate / graduate students to pursue careers in toxicology related areas (research, medical, regulatory, government and industry related career pathways); b) To network with other undergraduates / graduate doctoral students for advice and mentor- ing; c) To strengthen their CVs for better career pathways; and d) To increase awareness about toxicology related concepts in parent organization/school. We encouraged group members to attend all SOT 2020 related activities, such as the Virtual Undergraduate Education Program Webinar and the Online Undergraduate Research Presentations. Following these meetings, the group discussed their thoughts on the materials learned. As One-Health was the general theme for the UDP 2020 Program, the following three project topics related to this theme were suggested: Microplastics & Marine Environment, Heavy Metals & Sharks - food safety, Algal Bloom Toxins & Environmental Exposures. To address one of these topics a systematic evidence map approach was utilized. By participating in the project, students would: 1) learn the difference between systematic maps and systematic reviews, 2) develop a relevant PECO statement for the project’s scope and the corresponding research question, 3) learn of the tools available to help in the systematic mapping process, 4) learn the role of stakeholders within interdisciplinary projects, 5) conduct a systematic mapping project on a relevant and current topic in environmental health studies, and 6) suggest any updates to current policies or regulations relevant to their topic based on their findings. These project topics complement the overall context of our research projects conducted remotely with twice weekly career development activities over Zoom. We sought to develop an interactive session that informed 20 SURF students about lead toxicity. The training activity included four components: 1) didactic lecture on lead toxicity, sampling drinking water, and quantitation by ICP/MS, 2) community perspective on lead contamination and remediation, and 3) a simulated test of unknown lead levels using a Science Takeout® kit, and 4) sampling of student’s home drinking water and measurement of lead levels. Kits and sampling materials were mailed to participants prior to the session. The activity was held on Zoom and included interactive polling questions to ensure real-time comprehension. Samples from home testing were returned to Rutgers for ICP/MS quantification. Four weeks later, students participated in a second session to review lead levels in their drinking water and compare to local and national drinking water standards. Instructors also discussed causes of variability in heavy metal concentrations. Engagement in this activity was high with 90% of participants submitting samples for measuring of lead concentrations. Pre- and post-program self-assessments using 5-point Likert rating scales were conducted online. The ability of students to 1) simulate experiments at home (means: pre-1.4; post-2.3; p=0.02) and 2) conduct field testing and analyzing drinking water contamination (means: pre-2.1; post-3.1; p=0.004) was improved over the four weeks. Similar increases were observed in students’ understanding of the impact of environmental chemicals on the health of communities (p<0.0001) as well as the steps to test for heavy metal contamination in drinking water (p<0.0001). Taken together, this multifaceted training activity can improve understanding of environmental chemical toxicity as well as increase skills in field sampling. Supported by R25ES020721, T22ES007148, P30ES0505022, ULTR00317, U54AR055073 and the SOT and ASPET SURF Intern Programs.

3064 Breaking through the Noise: Virtual Training to Effectively Communicate Science


The ability to effectively communicate is a key requirement for scientists to disseminate their research. Due to the COVID-19 pandemic, the Rutgers Summer Undergraduate Research Fellowship (SURF) program adapted their training to a virtual 6-week format that included mentored research projects conducted remotely with twice weekly career development activities over Zoom. We sought to develop an interactive session that informed 20 SURF students about lead toxicity. The training activity included four components: 1) didactic lecture on lead toxicity, sampling drinking water, and quantitation by ICP/MS, 2) community perspective on lead contamination and remediation, and 3) a simulated test of unknown lead levels using a Science Takeout® kit, and 4) sampling of student’s home drinking water and measurement of lead levels. Kits and sampling materials were mailed to participants prior to the session. The activity was held on Zoom and included interactive polling questions to ensure real-time comprehension. Samples from home testing were returned to Rutgers for ICP/MS quantification. Four weeks later, students participated in a second session to review lead levels in their drinking water and compare to local and national drinking water standards. Instructors also discussed causes of variability in heavy metal concentrations. Engagement in this activity was high with 90% of participants submitting samples for measuring of lead concentrations. Pre- and post-program self-assessments using 5-point Likert rating scales were conducted online. The ability of students to 1) simulate experiments at home (means: pre-1.4; post-2.3; p=0.02) and 2) conduct field testing and analyzing drinking water contamination (means: pre-2.1; post-3.1; p=0.004) was improved over the four weeks. Similar increases were observed in students’ understanding of the impact of environmental chemicals on the health of communities (p<0.0001) as well as the steps to test for heavy metal contamination in drinking water (p<0.0001). Taken together, this multifaceted training activity can improve understanding of environmental chemical toxicity as well as increase skills in field sampling. Supported by R25ES020721, T22ES007148, P30ES0505022, ULTR00317, U54AR055073 and the SOT and ASPET SURF Intern Programs.
Genetic variation in cytochrome P450 (CYP) enzymes can alter drug metabo-

lism and in turn, the toxicity and safety of medications. Over the last
decade, clinical guidelines have been developed to use a patient’s genetic
profile to optimize drug therapy and avoid adverse side effects. The online
PharmGKB pharmacogenomics knowledge resource has annotated these
guidelines for clinical implementation of genotype-based drug dosing. We
sought to develop and evaluate an interactive, team-based activity to en-
geage high school students with annotated pharmacogenetic dosing guide-

lines. Due to the COVID-19 pandemic, the week-long Toxicology, Health
and Environmental Disease (THED) program for high school students was run on
a virtual platform and included 64 participants. This interactive pharmacoge-
netics activity lasted 90 minutes and focused on four components: 1) primer
on genetics, pharmacokinetics, and pharmacodynamics, 2) overview of the
PharmGKB website, 3) team-based case studies, and 4) student presentations
of clinical cases, including their dosing recommendations. Case studies fo-
cused on genetic variations in CYPs associated with clinical outcomes, includ-
ing depression (citalopram, CYP2C19), analgesia (codeine, CYP2D6), transplant-
tation (tacrolimus, CYP3A4), and HIV treatment (efavirenz, CYP2B6). Teams
were assigned one of the case studies to work on in Zoom-breakout rooms
using the PharmGKB database. Pre- and post-program self-assessment of
participants’ understanding of pharmacogenetic principles were conducted
online using 5-point Likert rating scales (means: pre-2.8, post-4.3, p<0.0001).
Evaluation of the activity was favorable with 86% of participants rating the
activity as ‘very good’ or ‘exceeded expectations’. We propose that an interac-
tive, team-based activity can be used to teach basic principles of pharmacog-
genetics and engage learners with online drug dosing resources. Supported by
T32ES007148, P30ES005022, UL1TR003017, and U54AR055073.

As toxicologists, we are ethically bound to reduce the numbers of animals
used in experiments, refine our methods to reduce suffering, and to use al-
teratives whenever possible. One recognized way of reducing animals is to
improve data analysis. The Bayesian approach to data analysis, which is now
computationally tractable, is one really good way to reduce the number of
animals. One way Bayesian approaches differ from traditional statistical
analyses is by using “prior information” - that is, information about the study
that is already out there. In toxicology, we tend to use the same vehicles, and
we also tend to use the same strains of model organisms in our studies. What this
means is that we, as a community, have a large repository of vehicle informa-
tion about our studies, much of it funded by taxpayers. By being able to tap
into these vehicle studies, we would much more precise estimates of animal
responses to vehicle - so much that we would need very few contemporane-
ous vehicles to be performed in any future study (note that we still need some
contemporaneous vehicle animals to ensure the study does not have signif-
ificant environmental or other unaccounted for effects). In this presentation I
will show, using simulations, how just having better precision in the vehicle
animals can lead to less uncertainty in the overall data analysis. Also, using the
Bayesian approach, we may be able to use prior information on the chemical
of interest, to obtain even better precision there, too. But for this to work, we
need open data standards, and for toxicologists to actually share their data.
I will also discuss ways from an applied ethics standpoint why we need open
data standards, and for toxicologists to actually share their data.

Bayesian Analysis and Data Availability to Improve Animal Welfare Ethics

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Since 2013, the Toxicology, Health, and Environmental Disease (THED)
program has been conducted at Rutgers University as a one-week summer camp
to open high school students interested in careers in environmental health,
toxicological science, and medicine. This program adopts a hybrid (labora-
tory-lecture) setting to teach core biomedical and toxicological principles.
In the summer of 2020, due to increased safety measures amidst the COVID-
19 pandemic and suspension of face-to-face instruction, THED underwent a
necessary, transformative shift to remote instruction. Students (n=64) par-
ticipated in a 4-day intensive online program consisting of various modules
that relayed concepts fundamental to the sciences. These included 1) funda-
mentals of research (laboratory safety and ethics, the importance of research
funding, and working together on a research team) and 2) life sciences and
toxicology (mechanisms of toxicity, selecting the appropriate model organ-
isms for a study, and gross and microscopic clinical presentations of disease).
Students received didactic instruction in one of two formats: 1) a standard
lecture format (individual learning topic, n = 7 or 2) a combination of lecture
and small group interaction (group learning topic, n = 10). Students com-
mpleted a survey before and after completion of the program that included a
self-valuation of their confidence in performing specific skills using a Likert
scale (Not Confident to Completely Confident). The number of students who
reported feeling completely confident, regarding a topic or task significantly
increased (p<0.001) after completion of the THED program when compared to
the pre-survey results for both individual learning (1.3±0.4 vs. 2.6±0.2) and
group learning topics (12.3±3.9 vs 39.9±1.8). Notably, the number of responses indicating complete confidence was significantly increased
(p<0.05) between the group learning and individual learning topics (39.9 ±
1.8 vs 7.0).

Hurricane Harvey was a category four storm that induced catastrophic rain-
fall-triggered flooding in the Houston metropolitan area. Multiple indus-
trial complexes released excess emissions due to unplanned shutdowns or
infrastructural damage as a result of the hurricane. Additionally, 13 of the
41 superfund sites within Harris County, Texas flooded. Public health con-
cerns were immediately raised regarding potential exposure to toxic chemi-
cals from the excess industrial releases and flooded superfund sites. Here we
focus on endocrine disrupting chemicals (EDCs), due to their high frequency
of detection, and since many of the superfund/industrial sites that flooded are
what is known or suspected of harboring EDCs. In collaboration with Baylor
College of Medicine and University of Texas-Houston, Oregon State University
deployed personal passive sampling wristbands immediately after the hur-
ricane to flood impacted geographic regions within Harris County (n=5). Par-
ticipants wore a wristband for seven days and completed a questionnaire
to retrieve their home flooding status and whether they participated in
flood clean up. This questionnaire was used to conduct an assessment of the
association between flooding and chemical exposure as well as to calculate an
Area Deprivation Index (ADI), which was used to assess a participant’s so-
cioeconomic status. We analyzed each wristband (n=173) for 1,530 chemi-
cals. Additionally, we compared the list of chemicals we analyzed to the TedX
list of potential EDCs (pEDCs), and the United Nations and Danish EPA list
of EDCs. With the exception of one pEDC similar exposure profiles existed
across participants, independent of home flooding or flood clean up status
(Wilcoxon Rank Sum and unadjusted odds ratios, p<0.05). The most profound
finding related to flood status is that participants who were living in a flooded
home at the time the study took place had increased frequency of detection
and concentrations found in wristbands multiple EDC and pEDCs compared to
participants whose homes flooded and moved after flooding. Additionally,
a high ADI (low socioeconomic status) was associated with the likelihood
of living in a home post-flood. When analyzed across geographic regions, par-
ticipants with the highest ADI who lived in the poorest neighborhoods had
higher levels of exposure to five EDCs and three pEDCs than participants with
low ADIs (Kruskal-Wallis, unadjusted odds ratios, p<0.05). Additionally
the lower income neighborhoods had significantly higher average sum concen-
trations of totals chemicals detected, EDCs, and pEDCs than the higher in-
come neighborhoods (Tukey-Kramer, p<0.05).
E-cigarettes are a significant public health concern, with over 12 million current e-cigarette users in the U.S., including 3 million high school students. E-cigarettes are often touted as an alternative to smoking traditional cigarettes and perceived as safer than cigarettes, but a growing body of research indicates that e-cigarettes can significantly affect the respiratory immune system. Increasing numbers of e-cigarette users, particularly youth, highlight a need for community engagement to increase science literacy and bridge the gap between scientists conducting e-cigarette research and the stakeholders who can benefit from these findings. To address this gap, we developed educational materials for K-12 audiences and healthcare professionals through collaborations with 1) the UNC Center for Environmental Health and Sustainability’s Community Engagement Core to develop data-based high school biology lessons and 2) the Mountain Area Health Education Center to develop an e-cigarette use assessment for primary care settings. Surveys collected (n = 92) following presentation of the biology lessons at state and national science educator conferences (n = 5) indicate that these sessions successfully deepened teachers’ knowledge about e-cigarettes (95% agree or strongly agree) and gave teachers ideas for integrating e-cigarette content into their instruction (95% agree or strongly agree), with the majority (76% yes, 23% maybe) reporting that they plan to integrate at least one lesson into their instruction. Feedback on the e-cigarette use assessment tool will be obtained via qualitative interviews with primary care practice staff members and a 12-member stakeholder group of young people aged 11-21 as part of an ongoing study. Stakeholder feedback and partnerships with scientists are critical to the success of such curricula and clinical tools to provide educational content that is real, relevant, and robust. Through these easy-to-use tools we have demonstrated effective ways in which scientists can engage in their communities to increase science literacy, thus help translate knowledge into action. Though these projects are specific to e-cigarette research, they can serve as examples for how scientists in all areas of toxicology can effectively engage the stakeholders in their research through community outreach activities.

**3070 The Role of Environmental Stressors on a UNC13A Single Nucleotide Polymorphism in *C. elegans* and Motor Neuron Degeneration: A Possible Cause of Sporadic Amyotrophic Lateral Sclerosis**

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that is highly fatal and is characterized by specific clinical manifestations and pathophysiology. Previous research suggests a gene-environment interaction may influence the development of sporadic ALS (SALS) which make up 90%-95% of all ALS diagnoses. A meta-analysis revealed that a single nucleotide polymorphism (SNP), rs12608932, located in an intron of UNC13A was associated with a greater risk of SALS development. The CRISPR/Cas9 system will be used to incorporate the UNC13A SNP in a novel *C. elegans* model using the unc-13 homolog A strain. It is hypothesized that the polymorphism rs12608932 in UNC13A in a *C. elegans* model exhibits characteristics of motor neuron degeneration like that of ALS after exposure to environmental stressors. An environmental stressor is expected to influence neurodegeneration in *C. elegans* by causing the onset of the phenotype. As we are commonly exposed to MeHg, Pb, and pesticides, it is imperative that their influence on genes are closely examined to create targeted therapeutics and genomewide association studies. It is expected that the novel *C. elegans* model proposed will be a sufficient model for further ALS studies and for understanding the role the rs12608932 SNP has in the development of the disease. Confirmation of this will allow the creation of targeted therapeutics for the UNC13A SNP in ALS, and genomewide association studies.

**3071 Methylmercury Induces Allodynia through Activation of Inflammatory Microglia in Spinal Cord and Subsequent Stimulation in Somatosensory Cortex of Rats**

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Methylmercury (MeHg) is an environmental poison and causes nerve dysfunction resulting from its neuropathy. The MeHg-induced neuropathy induces significant hypesthesia in the extremities, but in recently chronic pain has been observed mainly in the lower limbs centered on plantar of chronic phase patients with MeHg poisoning. In this study, we investigated the onset mechanism of neuropathic pain in MeHg poisoning using MeHg-intoxicated model rats. We demonstrated for the first time the MeHg induces a lower pain threshold “allodynia”, a symptom of neuropathic pain, in the lower limbs center on the plantar of chronic MeHg-intoxicated rats. After MeHg exposure, a significant decrease of pain threshold in the plantar was observed compared to the vehicle control. Histological and biochemical analyses revealed that nerve axon injury in the dorsal root cord and dorsal root nerve of the spinal cord, and the increased expression of inflammatory microglia in dorsal horn of the spinal cord, before the onset of allodynia in MeHg-intoxicated rats. Furthermore, it was found that the expression of thrombospondin-1, which is a marker of crosstalk between tactile and painful sensations, increased specifically in the somatosensory cortex. These results suggested that MeHg exposure could induce allodynia in the lower limbs of chronic phase patients with MeHg poisoning. In addition, it was indicated that the onset mechanism was caused by the stimulation of the somatosensory cortex by nerve injury of the dorsal root cord and dorsal root nerve of the spinal cord with inflammatory microglia, inducing the crosstalk between tactile and painful sensations there.
Lead (Pb) is a well-known neurotoxicant and environmental hazard. Recent experimental evidence has linked Pb exposure with neurological deterioration leading to neurodegenerative diseases such as Alzheimer’s disease (AD). To understand brain regional distribution of Pb and its interaction with other metal ions, we used synchrotron micro-XRF technique to map the metal distribution pattern and to quantify metal concentrations in mouse brains. Pb-exposed mice received oral gavage of 27 mg Pb/kg as Pb acetate (PbAc) once daily for 4 weeks; the control mice received Na acetate. Brain tissues were cut into slices and subjected to analysis. Synchrotron μ-XRF scans were run on the P06 beamline at the Deutsches Elektronen-Synchrotron (DESY) facility. Coarse scans of the entire brain were run to locate the cortex and hippocampus regions, after which scans with smaller step sizes of about 1 μm were run in these areas. The results showed that: a) Pb deposited in localized spots of <10 μm in the cortex region of both the exposed and unexposed samples, with ~3 times more of these spots in exposed samples than those in unexposed brain samples; b) selenium (Se) co-deposited with Pb and was significantly correlated with Pb in these spots; c) the total Pb signals in Pb-exposed brain slices were much greater than those in control brain slices. There was also evidence of strong correlation of Pb and Se in other regions. These results suggest that Pb ions are not evenly distributed throughout the entire brain structure, but rather tend to accumulate excessively in some localized spots in brain cortex. Se appears to co-exist with Pb. While the structural and chemical relationships between Se and Pb in localized spots remain unknown, Se seems likely to play a crucial role in Pb-induced neurotoxicity. Our findings call for further studies to investigate the relationship between Pb exposure and the deposition of Pb and other metal ions, and to understand the implication in the etiology of AD. Supported in part by NIEHS R01 ES027078.

3075 Activity of the Manganese Efflux Transporter SLC30A10 in Catecholaminergic Neurons Protects against Manganese-Induced Motor Deficits

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Elevated levels of the essential metal manganese (Mn) are neurotoxic and lead to an incurable movement disorder. The specific neuronal subtypes most effected by Mn are still unknown, hindering therapeutic progress. To understand Mn neurotoxicity and subsequent motor deficits, our lab studied the primary Mn efflux transporter, SLC30A10. Our recent work revealed that activity of SLC30A10 in the brain reduced Mn levels in the basal ganglia and thalamus during overexposure (Taylor et al., 2019). Here, we use various SLC30A10 knockout mice to selectively increase Mn levels throughout the brain (pan-neuronal/glial), in catecholaminergic neurons, or in GABAergic neurons and further elucidate the role of SLC30A10 in regulating brain Mn homeostasis. We found that pan-neuronal/glial SLC30A10 knockouts exhibited hypolocomotion that is exacerbated by a human disease-relevant Mn exposure regimen. HPLC studies revealed this hypolocomotion is not associated with changes in extracellular levels of striatal GABA, dopamine, or the dopaminergic metabolite DOPAC. However, catecholaminergic, but not GABAergic, knockouts also exhibited hypolocomotion. Thus, while elevated Mn may not lead to GABAergic or dopaminergic neurodegeneration, it may lead to dopaminergic dysfunction. Future work will use in vivo microdialysis to determine how Mn impacts dopaminergic function.

3076 Sensitivity and Specificity of MRI Markers of Manganese Brain Deposition

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Exposure to welding fumes leads to accumulation of manganese (Mn) in the brain. Since Mn acts as MRI contrast agent, MRI allows for a non-invasive estimation of increased brain Mn levels in vivo and is the main diagnostic criteria for current Mn intoxication. Several MRI markers have been used in the literature: The Pallidal Index (PI), a ratio of T1-weighted signal intensities in the globus pallidus (GP) and frontal white matter (FWM), and the physical property of R1 relaxation rate in different brain regions. Being less dependent on imaging parameters, the R1 is hypothesized to be more sensitive, however a formal comparison was never made. This study aimed to compare these two MRI markers’ sensitivity and specificity by performing a receiver operating characteristic curve (ROC) analysis. Both, the capability to distinguish between occupationally exposed subjects from unexposed controls, as well as sensitivity and specificity in distinguishing high exposed welders from low exposed welders were investigated. PI values were obtained from T1-weighted MRI data from a prior study on 70 subjects (42 male welders and 28 age-matched controls) recruited from a local manufacturing plant. R1 values for GP, FWM, and substantia nigra (SN) were calculated from whole-brain 3D R1 relaxation maps that were segmented into 192 ROIs using FreeSurfer. For each marker, PI, R1 in GP, FWM, and SN, an ROC analysis was performed in R to identify the area under the curve (AUC) and a threshold with the highest sensitivity and specificity for distinguishing welders from controls. Additionally, we separated our cohort into two subgroups, a high exposure (HEX) and low exposure (LEX) subgroups from FWM to SN, respectively. We found that the HEX had a maximum AUC of 0.78 for PI and 0.76 for R1 in GP, FWM, and SN. Our results seem to reinforce the hypothesis that R1 is more sensitive and specific to Mn intoxication than PI. Interestingly, the brain region that best discriminates between welders vs. controls and HEX vs. LEX changes from FWM to SN, respectively. Supported by NIEHS R01 ES020529.
Lead (Pb) is an environmental and occupational toxin that has been suspected of contributing to Alzheimer’s disease (AD) and related dementia. Our previous studies have established that chronic Pb exposure increases brain levels of beta-amyloid (Aβ) and parenchymal amyloid plaques, a pathological hallmark for AD, in APP transgenic mice. Interestingly, Aβ also exists in the walls of leptomeningeal and cortical arteries and in capillary of the cerebral amyloid angiopathy (CAA), which is observed in approximately 85%-95% of AD patients. However, it remained unclear whether and how Pb exposure was able to induce CAA. This study was designed to investigate if subchronic Pb exposure affected the leptomeningeal blood vessels as results of induced CAA in both leptomeningeal and hippocampal areas by using the CAA transgenic mice. Tg-SWDI mice (2 months of age) received daily ip. injection of 27 mg Pb/kg as Pb acetate for 4 weeks; at the end of Pb exposure, our immunohistochemical data revealed significantly induced leptomeningeal and subicular vascular amyloid stained with the Aβ antibody and thioflavin S, as compared to control mice that received daily injections of Na acetate. In another set of experiments, single ip. injection at the dose of 27 mg Pb/kg for 24 hrs significantly induced over-expressions of TGF-β1 and fibronectin, two risk factors of CAA in the leptomeningeal blood vessels of Tg-SWDI mice (n=3 for both Pb and control groups). Furthermore, subchronic Pb exposure for 4 weeks induced the hypo-perfusion in these transgenic mice by dynamic contrast-enhanced CT quantitation, possible resulting from CAA-mediated brain ischemia. These data support our hypothetical theory that chronic exposure to Pb, at levels currently believed to be subtoxic or safe, preferentially induces CAA by activating the TGF-β1 pathway in cerebrovasculature, which facilitates the binding of Aβ to the vascular structure, leading to pathogenic origins of AD and related dementia. Supported by NIH grants R01-E502708 and R21-A067923.

**Pathogenic Role of Subchronic Lead Exposure in Cerebral Amyloid Angiopathy and Alzheimer’s Disease**

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Methylmercury (MeHg) is a developmental neurotoxicant capable of causing cognitive and motor deficits in children. The presentation of motor deficits suggests that MeHg may act on muscle-derived targets in the developing neuromuscular system, and potentially the neuromuscular junction (NMJ). We previously conducted a genome-wide association study (GWAS) using Drosophila melanogaster, which revealed many neuromuscular-associated genes that accompanied a muscle phenotype of myospheres in the indirect flight muscles (IFM). By assessing morphological and functional phenotypes of adult structures formed during pupal metamorphosis, following larval exposure to MeHg, we explored the existence of muscle-derived MeHg targets that might act in a non-cell-autonomous manner at the NMJ. The IFM neuromuscular morphology was visualized using fluorescent reporter fly strains and immunostaining and neuromuscular function was assessed via eclosion and flight behavior. Using a strategy of modulating the activity of the Nrf2 antioxidant pathway in either muscle or neural lineages during development, we first demonstrate that protecting either muscle or neuron development moderates MeHg toxicity and protects IFM morphogenesis, seen by a reduction in myosphere number. This rescue in IFM development parallels an improvement in eclosion and flight ability. This finding indicates that both the developing muscle and motor neurons contribute to the MeHg-induced phenotypes and prompting us to explore a role for the NMJ. Neuroligin 1 (nlg1), an identified GWAS candidate, is a muscle-restricted, NMJ-associated factor and the heterodimeric synaptic partner of neurexin-1 (nrx1). Through RT-qPCR, we show that nlg1 expression, relative to other nlg and nrx members, is selectively repressed early in progeny of Pb-exposed flies. Muscle-specific overexpression of nlg1 partially rescues MeHg-mediated deficits in both eclosion and flight. These findings indicate that both muscle and neurons are targets of MeHg, the forming NMJ is a MeHg-sensitive component of the neuromuscular system, and highlights nlg1 as a candidate that mediates MeHg’s disruption of NMu development. Future research will extend investigations of nlg1 and its heterotetrameric protein interactions at the NMJ as targets of MeHg, which may underlie the etiology of MeHg induced motor deficits. Supported by R01E502708, R01 E5010219, and T32E5007026.

**Neuroligin 1 Expression Implicates Methylmercury: Effects on Drosophila Neuroligin 1 Expression Implicates Neuromuscular Junction-Specific Targets**


Cadmium (Cd) is a heavy metal that has been recognized as one of the most toxic environmental pollutants around the world. Increasing evidence suggests that Cd is also a neurotoxicant. However, the underlying mechanisms for its neurotoxicity are not completely understood. Our previous studies have demonstrated that Cd exposure can impair cognition, olfactory memory, as well as adult neurogenesis in mice. Since adult neurogenesis plays an important role in cognition and olfaction, to determine if these adverse effects of Cd can be mitigated by conditionally enhancing adult neurogenesis, we utilized the transgenic caMEK5 mouse strain we previously developed and characterized. This mouse strain enables us to genetically and conditionally stimulate adult neurogenesis by administering tamoxifen to induce expression of a constitutively active form of MEK5 (caMEK5) in adult neural progenitor cells, which can increase adult neurogenesis through the activation of the endogenous ERK5 MAP kinase pathway. In the current study, the caMEK5 mice were exposed to Cd (0.6 mg/L) through drinking water for 38 weeks. The Novel Object Location (NOL) test was conducted during the exposure period to detect the onset of impaired cognition in mice. At 17 weeks into Cd exposure, impaired hippocampus-dependent spatial working memory was found in Cd-treated mice and tamoxifen was administered to induce caMEK5 expression to activate adult neurogenesis. After that, a series of behavior tests were conducted at different time points to monitor cognition and olfaction in mice. Upon completion of the behavior tests, brain tissues were collected for the cellular study of adult neurogenesis. In this study, we found that Cd impaired hippocampus-dependent short-term spatial memory, contextual fear memory, and olfactory memory in caMEK5 mice. These deficits were rescued by the tamoxifen-induced caMEK5 expression. Furthermore, Cd exposure decreased the total number of BrdU+ cells and BrdU+ NeuN+ cells in the dentate gyrus of the hippocampus in mice, and tamoxifen treatment recovered the impaired adult neurogenesis in Cd-treated mice. Our study provides the first strong evidence for the direct link between Cd-impaired adult neurogenesis and Cd-induced impairments of cognition and olfactory memory.

**Inducible and Conditional Stimulation of Adult Neurogenesis Rescues Mice from Cd-Impaired Cognition and Olfactory Memory**

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The recent discovery of inherited autosomal recessive mutations of the manganese (Mn) influx transporter SLC39A14, have provided a clinical population to study Mn-induced neurological disease. These mutation carriers present with elevated blood and brain Mn concentrations and early-onset dystonia-parkinsonism that is not responsive to levodopa therapy. The availability of SLC39A14 knockout mice (KO) has allowed the characterization of the behavioral, neurochemical, and neuropathological resulting from elevated blood and brain Mn concentrations. Previously, we showed that SLC39A14 KO mice exhibit elevated blood and striatal Mn concentrations similar to the human disease and expressed motor function deficits measured by activity cages and rotarod relative to wild type (WT). Analysis of the nigrostriatal dopaminergic system also showed no significant changes in striatal dopamine (DA) concentrations or DA metabolites measured by HPLC-ECD. Striatal tyrosine hydroxylase immunohistochemistry of dopaminergic terminals did not change in KO mice. Furthermore, unbiased stereological cell counting of dopamine neuron density (Nv) and soma volume (Sv) in the substantia nigra pars compacta reveal no differences between KO mice relative to WT. Here we show, using in-vivo microdialysis in awake animals that striatal potassium-stimulated DA release was significantly reduced in SLC39A14 KO mice relative to WT. Further, in the tail suspension test SLC39A14 KO mice exhibited dystonic movements such as hindlimb clamping and truncal torsion. In addition, some of the SLC39A14 KO animals exhibited strabismus, tail curling, hindleg dragging and retropulsion. Together, these findings suggest that Mn-induced dystonia-parkinsonism is due to, at least in part, to the dysfunction of nigrostrial dopaminergic neurons but these neurons do not degenerate. SLC39A14 KO represents an excellent animal model to study the human disease and to further elucidate the behavioral, neurochemical, and neuropathological of Mn dystonia-parkinsonism.

**SLC39A14 Knockout Mice—A Genetic Model of Manganese-Induced Dystonia-Parkinsonism**

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Environmental factors have been associated with psychiatric disorders and recent epidemiological studies suggest an association between maternal lead (Pb$_2^+$) exposure and schizophrenia (SZ). Previous studies from our lab have shown that chronic early life Pb$_2^+$ exposure (CELLE) recapitulates specific neuropathological, dopaminergic and glutamatergic system changes present in the SZ brain. Glutamatergic signaling mediated by N-Methyl D-Aspartate Receptors (NMDAR) has been widely studied in SZ (Coyle, 2006). D-Serine, a co-agonist of the NMDAR is being explored as a potential therapeutic approach to treat the cognitive deficits associated with this psychiatric disease. D-Serine is synthesized by the enzyme serine racemase (SRR) and degraded by the enzyme D-amino acid oxidase (DAAO) and changes in the balance of these enzymes have been observed in SZ. In the present work, CELLE rats were used to assess gene expression of SRR and DAAO in various brain regions using a life course approach. Male and female CELLE rats had resulting blood Pb$_2^+$ levels averaging ±1.9 (control) or 20-25 µg/dL (Pb$_2^+$). Regional brain gene expression of SRR and DAAO were measured in pre-weaning (postnatal day 14), early adolescence (PN14), late adolescence (PN50), or at adult (PN120) male and female rats using quantitative real time RT-PCR. We found that Pb$_2^+$ exposure significantly increased SRR and DAAO gene expression in the striatum at PN14 (p=0.0005 and p=0.0074, respectively) and PN28 (p=0.0315, DAAO p=0.0316) compared to controls. At PN50, Pb$_2^+$ exposure increased SRR expression (p=0.016) but not DAAO expression was increased by Pb$_2^+$ exposure (p=0.03). No changes were observed for SRR at this age. Pb$_2^+$ exposure did not change SRR and DAAO gene expression in the frontal cortex of male and female rats at any age. These preliminary studies show that CELLE increases the gene expression of SRR and DAAO in an age-dependent fashion and suggests dysregulation of D-serine metabolism. Our findings suggest the potential role of environmental factors, such as Pb$_2^+$, as a risk factor for mental disorders, and the D-Serine pathway as a potential target for intervention. Further studies, analyzing gene expression changes in the hippocampus and analysis of D-serine concentrations are under way.

Chronic exposure to elevated levels of manganese (Mn) leads to a neurological condition referred to as manganism, presenting symptoms similar to those of Parkinson's disease. We have previously reported that Mn dysregulated glutamate transporter 1 (GLT-1 in rodents, excitatory amino acid transporter 2 (EAAT2) in human), leading to excitotoxic neuronal injury. Repressor element 1-silencing transcription factor (REST) exerted neuroprotection in several animal models as well as human brain. In the present study, we investigated if REST enhances EAAT2 function to protect dopaminergic neurons against Mn-induced excitotoxicity by impairment of GLT-1 in an in vitro astrocyte-neuron co-culture model. We have also tested if astrocytic REST deletion in brain striatal region exacerbates Mn-induced dopaminergic toxicity and motor impairment. The results revealed that the EAAT2 promoter carried REST binding site, RE1 consensus sites, and bound REST, enhancing EAAT2 promoter activity, mRNA and protein levels in astrocytes. Moreover, REST overexpression in astrocytes attenuated Mn-decreased EAAT2 promoter activity, mRNA/protein levels, and glutamate uptake into astrocytes. The astrocyte-neuron co-culture studies showed that REST overexpression in astrocytes attenuated Mn-impaired GLT-1 associated excitotoxicity, oxidative stress, and apoptosis in dopaminergic neuronal cells. Astrocytic REST in the brain striatal region was deleted by infusion of AAV5-GFAP-Cre-GFP vectors into the dorsal striatum of adult mice. After 3 weeks, mice were exposed to Mn (MnCl$_2$, 30 mg/kg, intranasal instillation, daily) for 21 days, followed by open-field and rotarod tests as well as assessment of REST, TH, and GLT-1 levels. Astrocytic REST deletion in striatum exacerbated Mn-induced impairment of motor deficits as well as decreases in dopaminergic TH and astrocytic GLT-1 mRNA/protein levels in striatum. These findings strongly suggest that astrocytic REST contributes to attenuation of Mn-induced neurotoxicity, at least in part, by enhancing EAAT2 expression and function, which mitigates excitotoxic neuronal injury.

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With occupational exposure levels to manganese (Mn) having decreased over the years, the effects of toxic Mn exposure shifted from a parkinsonian syndrome to more subtle cognitive and motor effects. In a previous study on 45 welders, we reported significant correlations between manganese exposure and fine motor function. The goal of this follow-up study was to assess changes in neuropsychological (NP) test performance over a time period of 3 years in a subset of 17 welders who continued to weld at the same welding factory. At each visit, welders completed 7 NP assessments: Finger Tapping Test (FTT), Grooved-Pegboard Test (GPT), WAIS-III Digit Symbol Coding (WSC), WAIS-III Digit Span (WDS), Naming Tests (NT), Trail Making Test (TMT), and Verbal Learning Test (VLT). Exposure assessment was repeated using personal air sampling at work, combined with modeling of the changes in exposure to assess cumulative lifetime exposure. Mn levels in toenail clippings were determined using ICP-MS as marker of exposure over the past year. A repeated-measure ANOVA was used to test for a significant change in both the exposure metrics and the raw NP scores between the two time points. Spearman Rank correlation tests between NP scores and Mn exposure were completed at each time point (TPA and TPB) for the 17 welders in the longitudinal study. Past-year exposure, as measured by toenail Mn levels, was significantly reduced (p<0.05), with changes in cumulative exposure being near significance (p=0.06). Repeated ANOVA found a significant decrease in the FTT score (p=0.023), but increases in VLT-mean and VLT-total scores (p=0.014 and p=0.003, respectively) over time. None of these changes was predicted by cumulative exposure at TPA. While FTT scores were found to significantly correlate with both cumulative exposure and toenail Mn at TPA, also in the small longitudinal cohort, these associations were not reproduced at TPB. Correlations of WDS and TMT with toenail Mn levels looked similar in TPA (p=0.01, rho=0.50), TBP (p=0.01, rho=0.47) and p=0.08, rho=0.46), but were no longer significant at TPB. While the small sample size remains a clear limitation of this study, these results may indicate that motor function is not reversible with decreasing exposure, while memory, as measured by the VLT scores, may be reversible. At both time points, subjects with higher Mn levels performed worse in the WAIS-III Digit Span and Trail Making, which are measures of attention/memory and visual scanning/cognitive flexibility, respectively. A longitudinal study with a larger sample size will be necessary to better understand the predictors of such changes, or to confirm consistent correlations with exposure. Supported by NIH R01 ES020529.

Methylmercury (MeHg) is a persistent environmental neurotoxicant that has potential adverse effects on neurodevelopment. Previous studies showed that developmental MeHg exposure damages brain functions that were unmasked after a silent period of years or decades. However, the underlying mechanisms of the late onset neurotoxicity associated with MeHg exposure at early stage are still unclear. In the current study, we established a Caenorhabditis elegans (C. elegans) model of developmental MeHg latent toxicity. Early larvae stage 1 worms were exposed with various doses of MeHg (0, 0.05, 0.5 and 5 µM) for 48 h. Their swimming behavior was recorded with the software WormLab for 300 s. Swimming speeds were compared at days 1, 5 and 10 adult stages. Worms with MeHg (0.05, 0.5 and 5 µM) demonstrated a significant decline in moving speed during swimming at day 10, but not day 1 or 5. Day 10 adult worms treated with MeHg showed a significant decrease in bending angle and frequency during swimming. Furthermore, their reduced moving speeds trended towards an increase. Dopamine signaling is involved in the regulation of worms’ moving speed. The C. elegans homologue gene of tyrosine hydroxylase, the rate-limiting enzyme for dopamine synthesis. The cat-2 mutant worms did not show a decline in moving speeds during swimming at day 10 adult stage. Two-way ANOVA analysis showed that there was a significant interactive effect between MeHg concentration and time on the moving speeds. Staining was performed extracellular dopamine levels by transporting dopamine back into the intracellular pool, establishing a feedback loop for the control of spillover of dopamine signaling. Analysis of moving speeds of worms with dat-1 deletion showed that the swimming speeds were further reduced after MeHg exposure. However, the effects of MeHg and dat-1 deletion were not synergistic, as the interactive term did not attain significance. Analysis of worm internal mercury levels showed that worms at adult stages experienced a marked elimination of the MeHg. Altogether, our results suggest that the latent decline in swimming speed by MeHg is modified by tyrosine hydroxy-
Methylmercury (MeHg) poisoning causes neurodevelopmental toxicity, with glutamatergic neuronal dysfunction being a hallmark of developmental exposures. However, it is currently unknown whether this neurotoxicity stems from change in neuron identity, neuronal dysfunction, or cell death. Therefore, we aimed to model persistent MeHg neurotoxicity for mechanistic study. Human-induced pluripotent stem cells (hiPSCs) were differentiated down a cortical glutamatergic lineage and exposed to either 0.1 or 1.0µM MeHg during the differentiation of either neoprogenitor cells (NP, D4-D10), intermediate progenitor cells (IP, D14-D20), or both (NP-IP). At D21 of differentiation, twenty-four hours after IP exposure, effects of MeHg exposure on differentiation were examined with qPCR and immunocytochemistry. The 1.0µM NP-IP exposure decreased mRNA expression of the deep layer cortical neuronal marker TBR1 and increased the expression of the radial glial marker FOXG1. The percentage of cells staining positive for the neuronal marker MAP2 was also significantly reduced. To determine if these changes were due to either a developmental delay or change in developmental trajectory, cells were further differentiated in the absence of MeHg until D38. Cell type and gene expression were then examined by single cell RNA sequencing (scRNA-seq), which identified 9 distinct clusters of cell types, most along the cortical development trajectory. The 1.0µM NP-IP exposure resulted in a persistent decrease in TBR1 expression and a corresponding decrease in the percentage of cells within subpopulations of the neuronal clusters. While FOXG1 expression was upregulated at D21, it was downregulated at D38. Conversely, the radial glial marker, PARX, was upregulated within the radial glial clusters. Ingenuity Pathway Analysis of significant expression changes showed that MeHg altered the expression of genes enriched within pathways implicated in neuronal development and metabolic homeostasis, such as mTOR, eIF2 and sirtuin signaling (p<0.05), across multiple cell clusters. These data suggest that MeHg may alter key pathways that regulate neuronal development and function, leading to a persistent alteration in cortical glutamatergic neuronal differentiation. NIH R01 ES07331, ABB, MA.

Manganese (Mn) is essential for life, including brain development, nerve and cognitivefunctioning. Several kinases of the insulin/IGF signaling cascade are Mn-dependent. Studies have shown that Mn activates kinases in the IGF pathway including mTOR and AKT which are well-accepted targets for Mn toxicity. As Mn is a known insulin mimetic, the overarching goal here was to identify direct molecular targets in insulin signaling pathway that are altered in Mn-dependent. Studies have shown that Mn activates kinases in the IGF signaling pathway. Studies have identified hpo-9/BTBD9 as a genetic risk factor for RLS. However, its molecular function remains largely unknown. Here, we present novel findings on the interaction between BTBD9, manganese (Mn) and insulin/insulin-like growth factor (IGF) signaling in Caenorhabditis elegans (C. elegans), mouse Neuro2a cells and humans. The nematode has a single BTBD9 mammalian homolog known as hpo-9. hpo-9 mutants were more sensitive to Mn-induced cellular stress and dopaminergic neurotoxicity, but not iron, copper or zinc toxicity. Mn exposure down-regulated hpo-9/BTD9 mRNA levels. However, in Mn levels were not altered, suggesting BTBD9 does not regulate total cellular Mn content per se; in contrast, Fe levels were significantly increased by hpo-9 over-expression. Additionally, non-toxic Fe pretreatment was able to protect from Mn-induced lethality in hpo-9 worms, but not the control. Moreover, expression of hpo-9/BTD9 restored the protection against Mn-induced toxicity and this protection was dependent on the forkhead box O (FOXO) protein. Furthermore, HPO-9 substantially increased FOXO levels and we identified protein kinase B (PKB) as the downstream target of HPO-9. These studies reveal a novel molecular mechanism by which BTBD9 mediates Mn-induced toxicity via the insulin/IGF signaling pathway and suggest Mn as a potential environmental risk factor for RLS.

**3092 Single Low Doses of Deltamethrin Cause ER Stress and Decrease Hippocampal Neurogenesis in Mice**

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Deltamethrin is a type II pyrethroid insecticide widely used in agriculture and homes for pest control. Previously, we reported that repeated exposure to 3 mg/kg deltamethrin for 30 days or 60 days caused marked reduction of adult hippocampal neurogenesis and impaired learning and memory in mice. Here, we sought to precisely identify dose-related effects of deltamethrin on hippocampal neurogenesis at different time points following single exposure. Adult male mice were given a single i.p. injection of 300 mg/kg BrdU at 24 h, 48 h, 7 d, or 14 d following a single oral exposure to 0, 0.3, 1, or 3 mg/kg deltamethrin and euthanized 2 h later to assess the acute and intermediate term effects of deltamethrin on the NSC population and proliferation. Data reveal that doses as low as 1 mg/kg of deltamethrin produced significant reduction in BrdU- and Ki67-positive cells in the dentate gyrus (DG) of the hippocampus as early as 48 h after exposure. Mice exhibited with decreased number of immature neurons (DCX-positive cells) started at 7 days after deltamethrin exposure. A significant induction of ER stress and activation of apoptotic signaling were also observed in the groups treated with 1 and 3 mg/kg of deltamethrin at 24 and 48 h following exposure. These data demonstrate that single exposure to very low doses of deltamethrin may cause disruption of neurogenesis and cognitive impairments in human. Supported by 1R01ES027481.

**3093 In Vitro Evaluation of a Candidate Pesticide Initiator of Expanded CAG Repeats in Huntington’s Disease**

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Huntington’s disease (HD) is a fatal neurodegenerative disorder deriving from expanded CAG cytosine, adenine, and guanine (CAG) repeats in the Huntingtin (HTT) gene. Healthy individuals tend to have anywhere from 4 to 15 CAG repeats on both copies of the gene whereas individuals affected...
by HD carry an expansion of 36 or more repeats in one copy. While HD is predominantly inherited, some cases, up to 4% or more, arise through sporadic CAG expansion. Environmental exposures may cause CAG repeat expansions, yet it is unknown which exposures contribute to repeat instability in trinucleotide repeat disorders such as HD. We previously demonstrated that pyraclostrobin, a prevalent oxidative stress-inducing fungicide, evokes gene expression signatures that are similar to those encountered in the HD patient. The objective of the current study was to determine if exposure to pyraclostrobin is capable of expanding CAG repeat length. Pyraclostrobin or a vehicle control was administered to an immortalized CAG-GFP reporter cell line as well as primary cortical cells and fibroblasts from the Q175 knockin mouse model of HD. Distributions of CAG repeat numbers were then measured using molecular fragment analysis. In addition to obtaining the main CAG repeat allele, an instability index (Lee et al., PMID: 20302627) was computed to investigate the occurrence of mosaic expansions existing in subsets of cells in each culture. The index can express either a widening or narrowing of the distribution of variability of CAG repeats around the main peak allele, and may thus be a more sensitive indicator of repeat instability compared to measuring the main CAG allele alone. We demonstrated that exposure to pyraclostrobin caused repeat expansion in the CAG-GFP reporter cell line and modestly increased the instability index. In contrast, mixed cortical cells from knockin mice did not show repeat expansion or altered instability upon pyraclostrobin treatment. Our results suggest that cell lines might be more prone to mitochondrial superoxide-induced trinucleotide repeat expansion compared to primary neuronal enriched cultures. Application of the instability index may prove to be a critical tool in understanding CAG repeat instability resulting from xenobiotic exposures.

Investigating the Influence of CHD8 Haploinsufficiency on Pyrethroid-Induced Developmental Neurotoxicity

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The considerable plasticity of the developing brain renders it exceptionally vulnerable to genetic and environmental perturbations. Autism Spectrum Disorder (ASD) is a common neurodevelopmental disorder with a strong but complex genetic component associated with key molecular pathways early in development. Yet, genetic risk factors seem insufficient to explain an increase in ASD prevalence over the past 15 years, raising the possibility that nonheritable risk factors are also at play. Exposure to exogenous agents during a critical developmental period has been suggested to contribute to ASD etiology. However, given the evidence on ASD heritability, environmental factors that play a role in ASD development likely influence mechanisms also involving some element of genetic susceptibility. Thus, there is an urgent need to identify mechanisms by which nonheritable factors may interact with susceptibility genes. In this study we investigate how haploinsufficiency in one of the most high confidence ASD risk genes, Chromatin Helicase DNA Binding Protein 8 (CHD8), influences pesticide-induced neurotoxicity. We report that Chd8 mutant mice demonstrate several abnormal phenotypes, including increased anxiety-like behavior in an elevated plus maze, decreased rearing movements in the open field, and hyper-sociability in a three-chamber test. These behaviors were altered in mice developmentally exposed to deltamethrin, an insecticide that functions by inhibiting sodium channel function. Using immunohistochemistry, we investigate differences in neuronal proliferation and neuronal maturation indicative of altered brain structures following developmental deltamethrin exposure. Further, we assess alterations in gene expression that may be responsible for the observed behavioral changes in Chd8 mutant mice with bulk RNA-sequencing of cortical tissue from postnatal day 5 mice and 1 year old mice exposed to deltamethrin.

Prior Exposure to Stress Hormone Exacerbates the Neuroinflammatory Response to the Nerve Agent Sarin and Pesticide Dichlorvos in a Mouse Model of Gulf War Illness

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Ongoing research into the underlying cause of Gulf War Illness (GWI) has repeatedly indicated a role for persistent aberrant neuroinflammatory signaling associated with neurotoxicant exposure; veterans with GWI had the potential for exposure to several neurotoxicants in theater. Our prior research using a GWI animal model has indicated that exposure to organophosphate acetylcholinesterase inhibitors (OP-AChEIs), such as the sarin (Sarin) and isopropyl fluorophosphate (DFP) or the pesticide chlorpyrifos, following administration of exogenous corticosterone (CORT) to mimic high physiological stress experienced in theater, results in robust neuroinflammation. In our effort to expand upon these results, we investigated if: 1) our DFP results were representative of nerve agent exposure; 2) CORT priming affected another OP-AChEI pesticide, dichlorvos (DDVP). In this study, mice were given CORT (200 mg/L) in the drinking water for 7 days followed by exposure to sarin (LD20=100 µg/kg, s.c.) or DDVP (20 mg/kg, i.p.). Cytokine expression was evaluated in the brain by qPCR (sarin and DDVP) and serum by multiplex ELISA (sarin) at 6 hours post-exposure. Like DFP and chlorpyrifos, exposure to sarin or DDVP following CORT resulted in a significant increase in brain cytokine mRNA. However, CORT + sarin only significantly increased three (IL-6, IL-12, and IL-17) of the 16 cytokines tested compared to DDVP alone. These data indicate that every known OP AChEI that veterans with GWI may have encountered in theater carries the potential to produce exacerbated neuroinflammation. The parallels between our observations with DFP and sarin validates the use of DFP as a surrogate to nerve agent in animal models. While these experiments focused on neuroinflammatory pathways, our prior GWI GWEx studies suggest in the literature to be affected by OPs (cholinergic neurotransmission, adenyl cyclase and oxidative stress) and assay negative and positive controls were also tested. Comparison of the holistic toxicological profile for each compound demonstrated that different OPs caused differential DNT phenotypes. Moreover, when compared with the mechanistic control compounds, the different OPs separated into distinct mechanistic clusters. Interestingly, the phenotypic profiles of adult versus regenerating planarians exposed to the OPs clustered differently, suggesting some developmental-specific mechanisms. Thus, this study provides new insight into how OPs differentially damage the developing brain. Supported by NIH grant R15 ES031354.

Comparative Analysis of the Mechanisms of Organophosphorus Pesticide Developmental Neurotoxicity in Freshwater Planarians

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Organophosphate pesticides (OPs) are a chemically diverse class of compounds used extensively. Epidemiological studies suggest that low dose chronic prenatal and infant exposures can lead to lifelong neurological damage and behavioral disorders. Inhibition of acetylcholinesterase (AChE) as the shared mechanism of acute OP neurotoxicity is well studied and used as the biomarker for OP exposure. However, OP-induced developmental neurotoxicity (DNT) can occur in the absence of significant AChE inhibition, suggesting alternative targets. Moreover, while different OPs can cause different adverse outcomes, most studies have focused on the most abundant OP, chlorpyrifos. Twenty-two “mechanistic control compounds” known to target pathways suggested in the literature to be affected by OPs (cholinergic neurotransmission, serotonin neurotransmission, endocannabinoid system, cytoskeleton, adenylylcyclase and oxidative stress) and assay negative and positive controls were also tested. Comparison of the holistic toxicological profile for each compound demonstrated that different OPs act through different DNT phenotypes. Moreover, when compared with the mechanistic control compounds, the different OPs separated into distinct mechanistic clusters. Interestingly, the phenotypic profiles of adult versus regenerating planarians exposed to the OPs clustered differently, suggesting some developmental-specific mechanisms. Thus, this study provides new insight into how OPs differentially damage the developing brain. Supported by NIH grant R15 ES031354.

Chlorpyrifos and Δ9Tetrahydrocannabinol Exposure and Effects on Parameters Associated with Obesity

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The use of medicinal and recreational cannabis (main component Δ9Tetrahydrocannabinol (Δ9THC)) has increased globally by 60% from 2010-2019 (>200 million in the US smoke cannabis: 2019). Obesity in the US has also rapidly increased to 42% in adults (2017-2018) with 20% prevalence in adolescents (2019). Further, chlorpyrifos (CPF), a neurotoxic organophosphate pesticide is used on cannabis plants. Both CPF and Δ9THC affect the endocannabinoid system (ECS), the central nervous system (CNS) reward system, the neuroendocrine, adipogenesis, metabolism, and gut microbiota, which, if disrupted, could lead to increased risk for obesity and related diseases. CPF inhibits EC breakdown at neural
synapses, thus inhibiting release of neurotransmitters, where, $\Delta^9$THC acts as an EC receptor agonist at synaptic terminals. To examine the effects of each compound on parameters related to obsesogenic activity, a literature search was performed with PubMed (Abstract Sifter), Google Scholar and other resources for CPF and $\Delta^9$THC effects on body weight, food intake, adiposity, lipids, glucose, insulin, inflammation, oxidative stress and gut microbiota. Out of 15000 articles, 37 were selected for a complete review, based on data for male rodents exposed to CPF or $\Delta^9$THC during gestation, perinatal/preweaning, weaning, adolescence and adulthood. Results showed effects from treatment during adolescence in common with CPF and $\Delta^9$THC (decreased body weights, increased triglycerides and cholesterol and inflammation). While dose-related body weights were decreased at all life stages for $\Delta^9$THC, they were decreased only in adolescence for CPF. Other CPF treatment life stages showed dose-related weight increases; a concerning result during critical stages when the developing young may receive second-hand exposure to both chemicals and adolescents may begin cannabis use. Both CPF and $\Delta^9$THC showed increased glucose, triglycerides and inflammation and decreased total cholesterol when treated in adulthood. Dose-responses were also seen with CPF as increased adiposity and glucose, with insulin and lipoprotein decreased, where $\Delta^9$THC had lipidoprotein effects. Effects observed between the two chemicals had little concordance, which may be due to the fact that the results were from labs using different inbred strains (12), experts in different domains that time and the ability of LNFPIII to modulate these effects. Over the course of 15 days, male C57BL/6J mice received daily exposure to the neuro-prophylactic pyridostigmine bromide, insect repellent DEET and corticosterone. On day 15, mice received a single injection of the sarin surrogate diisopropylfluorophosphate. LNFPIII treatment began 7 months post-GWI exposure and continued until study completion at 11 months. Of the data analyzed to date, 11-months post-GWI exposure and 4 months post-LNFPIII treatment, gross brain weights were decreased by GWI. In the dorsal (dH) and ventral (vH) hippocampus, areas important in learning/memory and regulating stress/ emotion, respectively, qRT-PCR analysis revealed brain region-specific increases in inflammatory cytokines (i.e. IL-6) and alterations in neurotranspecific factors (i.e. NGF, BDNF, and CNTF) that were modulated by LNFPIII. In the dH, increases in IL-6 by GWI were reduced by LNFPIII treatment, suggesting LNFPIII may aid in reducing GWI neuroinflammation. Further, dH levels of BDNF and NGF were increased by LNFPIII in mice with prior GWI exposure. Similar trends were also present in the vH, where numerical GWI-induced reductions in growth factor levels, particularly CNTF, were eliminated by LNFPIII. These results suggest that LNFPIII treatment may not only be anti-inflammatory, but also neurogenic and/or neuroprotective in brain regions important for memory and mood function. This research is supported by the Department of Defense grant W81XWH-16-1-0586 to NMF.

# 3098 Long-Term Neurobiological Alterations Caused of Gulf War Illness-Related Chemicals in Mice Are Ameliorated by a Delayed Treatment with the Immunotherapeutic LNFPIII


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Chemical overexposures during the 1990-1991 Gulf War (GW) are attributed to Gulf War Illness (GWI), a complex illness with many neurological symptoms associated with immune system disruption affecting roughly 30% of GW veterans. Our previous studies utilizing an established GWI model revealed selected motor, mood, and cognition/memory deficits at 8-10 months post-GWI related exposure. Many of these effects were eliminated with a novel immunotherapeutic, lacto-N-fucopentaose III (LNFPIII). Here, we characterized the long-term effects of GWI-acute alterations in parameters that occurred and the ability of LNFPIII to modulate these effects. Over the course of 15 days, male C57BL/6J mice received daily exposure to the neuroprophylactic pyridostigmine bromide, insect repellent DEET and corticosterone. On day 15, mice received a single injection of the sarin surrogate diisopropylfluorophosphate. LNFPIII treatment began 7 months post-GWI exposure and continued until study completion at 11 months. Of the data analyzed to date, 11-months post-GWI exposure and 4 months post-LNFPIII treatment, gross brain weights were decreased by GWI. In the dorsal (dH) and ventral (vH) hippocampus, areas important in learning/memory and regulating stress/ emotion, respectively, qRT-PCR analysis revealed brain region-specific increases in inflammatory cytokines (i.e. IL-6) and alterations in neurotranspecific factors (i.e. NGF, BDNF, and CNTF) that were modulated by LNFPIII. In the dH, increases in IL-6 by GWI were reduced by LNFPIII treatment, suggesting LNFPIII may aid in reducing GWI neuroinflammation. Further, dH levels of BDNF and NGF were increased by LNFPIII in mice with prior GWI exposure. Similar trends were also present in the vH, where numerical GWI-induced reductions in growth factor levels, particularly CNTF, were eliminated by LNFPIII. These results suggest that LNFPIII treatment may not only be anti-inflammatory, but also neurogenic and/or neuroprotective in brain regions important for memory and mood function. This research is supported by the Department of Defense grant W81XWH-16-1-0586 to NMF.

# 3099 Neurotoxic Mechanisms of a Sarin Surrogate beyond Acetylcholinesterase Inhibition

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One of the most pressing concerns with treating acute OP poisoning is that the current U.S. approved oxime, pralidoxime (2-PAM), cannot efficiently cross the BBB, thus failing to protect the brain against damage and subsequent long-term cognitive and behavioral deficits. Understanding the different mechanisms affected by OP exposure is important in developing and improving these neurotransmitters. Our laboratory developed a series of substituted phenoxyalkyl pyridinium oximes (US patent 9,227,937) that have been shown to provide central neuroprotection against OP exposure through the preservation of neuronal and glial structures in a rat model whereas 2-PAM did not. The purpose of this study was to further investigate additional neurotoxic mechanisms of the sarin surrogate, nitrophenyl isopropyl methylphosphonate (NIMP), as potential targets for neuroprotection by these brain-penetrating oximes. Utilizing activity-based protein profiling and a HPLC enzymatic assay in rat forebrains, FAAH was revealed as a target of NIMP and the lead oxime, Oxime 2B, showed increased enzymatic properties via FAAH inhibition. Also, by measuring the apoptotic activity of active caspase-3 and the concentration of necrotic receptor-interacting serine/threonine-protein kinase 1 (RIPK1) using an enzyme linked immunosorbent assay at 24 hours post lethal exposure, NIMP was found to cause necrosis in rat hippocampi and pallidum cortices. There were negligible amounts of active caspase-3 detected at the same time point, suggesting NIMP caused no apoptotic activity in the indicated brain regions. Further studies are needed to expand on these findings; however, these results suggest that FAAH and the necrotic marker RIPK1 are secondary targets of NIMP providing additional therapeutic targets for our novel oximes. Support NIH U01 NS107127.

# 3100 Lacto-N-Fucopentaose-III (LNFPIII) Ameliorates Acute Aberrations in Hippocampal Synaptic Plasticity and Transmission in a Gulf War Illness Animal Model


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Gulf War Illness (GWI) is a chronic multisymptom condition that affects a third of the Persian Gulf War veterans and presents with musculoskeletal pain, fatigue, and cognitive dysfunction. While the precise etiology of GWI is unknown, the onset of GWI is linked with overexposures to neurotoxic insecticides such as permethrin and organophosphate pesticides, such as pyridostigmine bromide (PB). There are currently no approved neuroactive therapeutics available for the treatment of GWI. The present study investigated the efficacy of a novel immunomodulatory agent, lacto-N-fucopentaosae-III (LNFPIII), in ameliorating acute neurophysiological deficits observed in a GWI mouse model. Male C57BL/6J mice were concurrently exposed to 0.7 mg/kg PB (i.p.) and 200 mg/kg (i.p.) once per day for 10 days. Control mice received DM50 vehicle instead of PB-PM. Subsets of vehicle and PB-PM-exposed animals were also coadministered carrier vehicle (vehicle-vehicle, PB-PM-vehicle) or LNFPIII (vehicle-LNFPIII, PB-PM-LNFPIII) treatment daily during the 10 day exposure period. 48 h after PB-PM exposure, dorsal (dH) and ventral hippocampal (vH) slices obtained from PB-PM-vehicle animals exhibited impaired basal synaptic transmission whereas long-term potentiation (LTP) magnitude was unaffected. Concurrent LNFPIII treatment enhanced dH and vH basal synaptic transmission in vehicle-treated animals. Notably, dH basal synaptic transmission deficits detected in PB-PM-exposed animals were ameliorated by LNFPIII treatment and dH LTP magnitude was also elevated. Key vH inflammatory cytokines were unaffected by PB-PM exposure; however, LNFPIII treatment numerically enhanced vH BDNF levels, an effect that was particularly pronounced in PB-PM-LNFPIII animals. Dorsventral-specific effects of PB-PM exposure and LNFPIII treatment on hippocampal synaptic plasticity and transmission provides mechanistic insight into the deleterious and beneficial effects of these agents, respectively. Importantly, LNFPIII coadministration during PB-PM exposure ameliorated short-term deficits in dH synaptic transmission and elevated dH LTP magnitude, suggesting that LNFPIII may be an efficacious treatment for neurogenic dysfunction observed in GWI, if it is effective in the long-term as well. Support: Department of Defense (CDMRP W81XWH-16-1-0586 to NMF, DAH, and JJW).

# 3101 Acute Intoxication with Diisopropylfluorophosphate (DFP) Decreases Synaptic Density in the Hippocampus of Adult Male Rats

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Acute intoxication with organophosphate (OP) cholinesterase inhibitors can trigger life-threatening status epilepticus (SE). Current medical countermeasures can prevent death from OP-induced SE, but do not effectively protect against chronic morbidity. The pathogenic mechanisms of these chronic effects remain unknown but are hypothesized to involve altered synaptic connectivity. Testing this hypothesis involves quantifying synaptic density over time. Histologic techniques are considered the gold standard for quantifying synaptic density in brain sections; however, these techniques are time-consuming and do not allow for longitudinal assessments of synaptic density.
over time in the same animal. In vivo PET imaging overcomes such limitations. The molecule \(^{18}F\)UCB-H specifically binds synaptic vesicle glycoprotein 2A (SV2A), a transmembrane protein expressed in synaptic vesicles, and has recently been developed as a PET probe for imaging synapses. The goal of this study was to determine whether synaptic density measured by PET correlates with ex vivo Golgi staining and immunohistochemical (IHC) synaptic metrics. Synapses were assessed by counting dendritic spines on Golgi stained neurons, and immunohistochemically by fluorescently labeling presynaptic SV2A, postsynaptic PSD95, and dendritic MAP2. Adult male Sprague-Dawley rats (200-250 g) were administered a single dose of DFP (4 mg/kg s.c.) or vehicle (saline) followed by atropine sulfate (2 mg/kg i.m.) and 2-pralidoxime (25 mg/ kg i.m.) 1 min later. Animals underwent in vivo MRI (for anatomic registration) and PET imaging at 10 and 28 days post-intoxication with a subset of animals euthanized after each time point to collect brain tissue. Hemisected brains were processed for Golgi staining and IHC. Our results indicate a significant decrease in \(^{18}F\)UCB-H uptake in the hippocampus following acute DFP intoxication, which appears dependent on the severity of DFP-induced seizures. Initial histological assessments indicate similar outcomes. Collectively, these observations demonstrate that acute OP intoxication causes a loss of SV2A binding sites consistent with an overall reduction in synapse density within the hippocampus and further suggest SV2A PET as a viable approach for assessing synaptic density in preclinical models. Funding provided by NIH CounterACT (USA NS079202).

### 3102 The H63D Variant of the Homeostatic Iron Regulator Protein (HFE) Protects against Paraquat-Associated Nigral Neurotoxicity in Agricultural Workers


Paraquat is an herbicide and oxidative neurotoxicant used widely in the United States. Its use is controversial, however, as studies have found paraquat exposure to be associated with development of Parkinson’s disease (PD), a neurodegenerative disorder characterized by the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc). Animal studies indicate that iron genetics may influence SNc susceptibility to paraquat. We previously found that the murine homologue to the H63D variant of the human homeostatic iron regulator (HFE) protects against paraquat-associated SNc neurotoxicity in mice. In this study, we used diffusion tensor imaging (DTI), an MRI-based neuroimaging technique sensitive to PD-related microstructural changes, to test whether H63D similarly protects against paraquat-associated SNc neurotoxicity in humans. This question is clinically relevant as H63D is carried by approximately one quarter of the U.S. population. HFE genotypes were determined in paraquat-exposed workers (16 HFE wildtypes; 9 H63D heterozygous carriers) and unexposed controls (21 HFE wildtypes; 18 H63D heterozygous carriers). DTI metrics of fractional anisotropy (FA) and mean diffusivity (MD) were obtained alongside serum iron panel measurements and compared between groups. Among unexposed subjects, HFE wildtypes and H63D carriers showed similar SNc FA and serum iron panel measurements. In wildlife, paraquat exposure was associated with significantly lower SNc FA and no change in serum iron. In H63D carriers, paraquat exposure was associated with no change in SNc FA but an approximately 40 percent increase in serum iron over all other groups. There were no MD differences between groups. Interestingly, there was a significant positive correlation between serum iron and SNc FA among paraquat-exposed but not unexposed subjects. Preserved SNc FA suggests that H63D may protect against paraquat-associated nigral neurotoxicity in humans, a possible gene-environment interaction in PD etiology. Future studies should examine potential mechanisms underlying this gene-environment relationship due to the high prevalence of H63D and the increasing burden of PD and related neurodegenerative diseases.

### 3103 Dichlorodiphenyltrichloroethane Exacerbates Tau Protein Toxicity in Caenorhabditis elegans

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Dichlorodiphenyltrichloroethane (DDT) is a persistent organic pollutant which, despite its ban in 1972, is present in the blood of most Americans to this date. Observational epidemiological studies have linked exposure to DDT with increased risk of Alzheimer’s disease (AD). We investigated whether DDT can exacerbate AD-related pathology using a transgenic C. elegans strain that expresses a mutant tau protein fragment that is prone to aggregation. Exposure to 3μM DDT resulted in internal levels of 0.5 pg (SD: 0.01) p,p’-DDT per worm and 0.1 pg (SD: 0.01) of its metabolite p,p’-DDE. We found that exposure to 3μM DDT significantly restricts growth in the transgenic strain more than it does in wildtype worms. Further, DDT significantly lowered mitochondrial respiration rates in both strains, as determined through Seahorse XF96 extracellular flux analysis (basal respiration in transgenic strain: 9.3 pmol oxygen/minute/worm (SEM: 0.7); vs with DDT: 5.8 pmol oxygen/minute/worm (SEM: 0.6), p < 0.05). High-resolution metabolomics (HRM) analysis using the whole worm showed reduced levels of several amino acids and an increase in adenosylselenohomocysteine in both strains exposed to DDT. DDT exposure in the transgenic strain significantly increased the amount of time spent curling, determined using the CeleST computer vision software, and showed altered TCA cycle metabolism, determined through HRM. The curling phenotype has been previously associated with mitochondrial dysfunction. Surprisingly, developmental exposure to DDT mildly rescued the shortened lifespan of the transgenic strain (mean lifespan: 8.3 days (SEM:0.8) vs with DDT: 11.8 days (SEM:1.1), p < 0.05), suggesting a mitohormetic effect of the “double-hit” from DDT and aggregating tau protein or an antagonistic effect which could ultimately turn on lifespan extension pathways. Our data suggest that exposure to DDT likely exacerbates the mitochondrial inhibitory effects of aggregating tau protein in C. elegans.

### 3104 Epigenetic Modifications in a DFP-Based Rat Model for Gulf War Illness

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Approximately 1/3rd of the returning soldiers from the First Gulf War suffer from a chronic multi-symptom condition known as the Gulf War Illness (GWI), chief among which are symptoms of fatigue, pain, along with mood and memory impairment. While the causes for GWI are known, the persistence of GWI is still poorly understood. Epigenetic mechanisms respond to external stimuli such as the environment and experiences, and the effects of such exposures can become embedded in the genome to produce long-lasting changes in cellular regulation. Epigenetic processes include chemical modifications including DNA methylation and histone acetylation and deacetylation. DNA methylation changes have been reported in blood samples of GWI veterans, however, the role of DNA methylation and histone modifications in the brain is not fully understood despite its importance as a major epigenetic mechanism for neurological disorders. Male Sprague-Dawley rats (3-m, n=30) were exposed to DFP (0.5 mg/kg s.c., 5-d) or ice-cold PBS and, 6-m later assessed for mood and memory function using a battery of rat behavioral assays. Rats were then sacrificed and brains harvested for protein studies and epigenetic analyses. Measurement of the percentage of DNA methylation (%5mC) revealed significant hypomethylation in GWI rats compared to age-matched control rats (p<0.05, n=6 rats, t-test). Antibody-specific western blotting studies revealed a significant upregulation in hDAC1 protein in GWI rats compared to age-matched control rats along with a significant increase in acetylated histone H3K9 in the hippocampus of GWI rats (p<0.05, n=6 rats, t-test). A locus-specific study of this epigenetic modification using chromatin immunoprecipitation (ChIP) revealed decreases in H3K9ac at the Bdnf promoter adjacent to exon 4 and a subsequent significant decrease in Bdnf protein levels in GWI rat hippocampus compared to age-matched control rats (n=8 rats/condition, t-test, p<0.05). Our studies indicate a role for epigenetic changes in the persistence of GWI symptoms and provides insights into the molecular mechanisms for GWI pathogenesis and novel targets for developing effective therapies for GWI behavioral symptoms.
In Vitro Study on the Effects of Combined Exposure to Chlorpyrifos or Chlorpyrifos Oxon on Provenofos Metabolism and Acetylcholine Esterase Inhibition
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Organophosphates (OPs) such as provenofos (PFF) and chlorpyrifos (CPF) have been extensively used in agriculture and their residues have been detected together in various fruits and vegetables. Consequently, consumers can be concurrently exposed to these OPs and insight in adverse effects upon combined exposure is desirable. The present study evaluated the effects of CPF and its metabolite CPF oxon (CPO) on the in vitro metabolic conversion and acetylcholine esterase (ACHE) inhibition of PFF. Metabolic interactions were evaluated using human liver microsomes, cytosol, and plasma and the results obtained indicate that both CPF and CPO inhibited PFF metabolism to its main metabolite (BCP) in a non-competitive manner, with K values of 120, 256 and for 473 μM, respectively, for CPF, and 80, 63 and 326 μM, respectively, for CPO. A previously published physiologically based kinetic (PBK) model of CPF and CPO was used to estimate CPF dose levels that are required to reach hepatic and plasma concentrations amounting to the K values. These analyses indicate that at relevant human exposure levels, CPF is not expected to interact with PFF metabolism. Concentration-dependent inhibition of human (recombinant) ACCHE was determined in vitro for CPF, CPO and for PFF/CPO combinations. BMCi values for ACCHE inhibition by PFF and CPO amounted to 826 and 5.0 nM, respectively, and combination studies suggest that competition effects follow the concept of dose addition. Altogether, this study indicates that at relevant dietary exposure to CPF and PFF, no toxicokinetic interactions are expected, and that ACCHE inhibition upon combined exposure to these OPs follows the concept of dose addition, supporting a toxic equivalency factor (TEF) or relative potency factor (RPF) approach for assessing combined exposure to these OPs.

Cytoskeleton Rearrangement Induced in RAW264.7 Cells by Exposure to Organophosphate Compounds

Organophosphate pesticides (OP) such as malathion, are well-known neurotoxic compounds with anticholinesterase activity. However, studies suggest that OPs interact with other molecular targets, including cytoskeleton proteins. Cytoskeleton participates in different cell functions such as phagocytosis, cell migration, and vesicular traffic, and its alteration can explain the impaired immune responses and neurotransmitter defects reported after OP exposures. In vivo and in vitro models, OPs metabolism leads to the formation of the non-specific metabolites dialkyl phosphates (DAPs), that can exert genotoxicity and immunomodulatory effects, suggesting that they could participate in OP-associated toxic effects. Here, we evaluated the effect of malathion and its DAP metabolites: dimethyl diithiophosphate (DMTDP), dimethyl thionophosphate (DMTP), dimethyl sulfoxide (DMSO) and dimethyl phosphate (DMP) on actin polymerization and cytoskeletal arrangement of murine macrophage RAW264.7 cell line. We observed that malathion and its DAPs reduced the actin polymerization rate at 0.01 μM, but only DMP reduced the total polymerized actin in an acellular polymerization assay. On RAW264.7 cells, we observed by confocal microscopy that malathion, DMTDP, and DMP metabolites (0.01 μM) induced cell polarization with elongated morphologies characterized by pseudopod and some filopodia around the poles of the cells after two hours of treatment. DMP also affects the tubulin cytoskeleton organization. The results suggest that malathion and DAP metabolites can directly alter actin dynamics and modify the cytoskeleton organization on macrophages. These alterations could lead to impaired immune responses and the development of diseases related to OP chronic exposure. Financing: CONACyT grant number 153468.

Utilizing a Comparative Thyroid Assay Decision Framework: A Risk-Based Approach to Prevent Unnecessary Animal Testing

Thyroid function and hormone regulation are particularly important for pregnant mothers, developing fetuses, and young children. To address whether thyroid function could be impacted during development by agrochemical exposure, the United States Environmental Protection Agency (USEPA) has requested further evaluation of agrochemicals that show key indicators of thyroid toxicity using the comparative thyroid assay (CTA). The CTA can use over 3000 rats to investigate potential thyroid related hazards to pregnant dams and offspring. Nevertheless, if thyroid effects occur at doses above the human health protective point of departure (POD), which are used for conducting risk assessment, then CTA data is deemed unnecessary for the evaluation of human health risk assessment (HHRA). A CTA decision framework was used to determine the need to conduct the study for three case-example agrochemicals which received a data call-in for this assay: Dithiopyr, a substituted pyridine herbicide used for non-food purposes, and the spinosyns spinosyn D and spinetoram, insecticides used to control a variety of pests. The decision framework considered the use scenario, potential human exposures, thyroid specific NOAEL-LOAEL gaps in already existing studies, exposure level to thyroid NOAEL/LOAEL gaps, and relevant uncertainty factors. Bench-mark dose (BMD) modeling was utilized to aid quantitative comparison across relevant studies and endpoints. The present study evaluated the exposure goals are met.

Mancozeb Exposure Results in Disruption of Mitochondrial Complex III and IV Activity in Transformed Human Colon Cancer Cells
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Mancozeb, a fungicide, is a member of the ethylene bisdithiocarbamate pesticides. The ethylene bisdithiocarbamate pesticides, a subclass of the dithiocarbamate pesticides, are mainly used in agriculture as fungicides on a variety of crops. The structure of Mancozeb is comprised of an ethylene bisdithiocarbamate backbone complexed to the metals manganese and zinc. Ingestion of food containing pesticide residue and occupational exposure are common routes of Mancozeb exposure. Previous studies in the literature have established Mancozeb as a mitochondrial toxicant. The purpose of this study is to investigate the effect of Mancozeb on the activity of specific mitochondrial enzymes of the electron transport chain. Previous work in our laboratory has shown that Mancozeb exposure results in cytotoxicity, induction of apoptosis and necrosis, and decreased Complex I, II, and IV enzyme activities. Current experiments utilized kinetic assays to evaluate and compare the effect of Mancozeb exposure on the activity of mitochondrial enzymes, specifically Complex III and IV. Mitochondria were isolated from HT-29 cells treated with Mancozeb (60-100 μM) for 24 hours. Complex V activity was used to confirm that viable mitochondria were isolated. Activity of Complex V enzyme was significantly decreased at 60 μM Mancozeb. Complex III enzyme activity was significantly decreased at 60 μM and 100 μM Mancozeb. These results, in combination with previous experiments, indicate that HT-29 colon cancer cells exposed to Mancozeb have decreased activity of all mitochondrial complex enzymes.

Acute and Long Term Exposure Study of Human Metabolism of Organophosphorus Compounds
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Although the use of organophosphorous pesticides (OPs) has diminished in recent years, OP poisoning still accounts for more than 100,000 deaths a year, making it a particularly relevant toxicological issue. The toxicity of acute OP exposure is due to the covalent inhibition of acetylcholinesterase resulting in muscle contractions, blurry vision, seizures, respiratory failure and death. Additionally, chronic low-dose exposure to OPs has also been implicated in a number of toxicological symptoms. In spite of the relevance of OP poisoning, little is known about the human metabolism of OPs, particularly any differences in metabolism that may exist between acute and low-dose exposures. This study aims at understanding the human metabolism of OPs using human liver microsomes (HLMs) at different OP concentrations to simulate the acute and low-dose exposure regimes. 1D-NMR was used for determining the rate of metabolism and structure elucidation of the metabolites at high OP concentrations (acute exposures) whereas mass spectrometry was used to assess
metabolism at lower concentrations (low-dose exposures). Twenty structurally diverse OPs were selected and used in the studies. For the acute exposure regime, the OPs were incubated with HLMs for up to five days during which $^{31}P$, $^{31}P$, and $^{31}P$ (if present) NMR data were recorded. For example, disopropyl fluorophosphate (DFP) was incubated with HLMs for five days with spectra recorded every two hours. The duration of the incubation and time points recorded depended on the rate of metabolism of the OP with an expectation that at least 95% of the original OP should be metabolized. The integral values from the $^{31}P$ NMR spectra were used to determine the clearance rates and all NMR data were utilized in structure elucidation of the metabolites. For DFP, analysis of the NMR spectra suggested the formation of disopropyl phosphate and disopropyl fluorophosphates as the main metabolites. Similarly, mass spectrometry was used to determine the clearance rates at lower concentrations by quenching the reaction at various time-points followed by quantitative LC-MS/MS analysis for rate determination. Based on the results obtained, we suspect that similar enzymes act on these structurally divergent chemicals, hydrolyzing comparable leaving groups from the OPs. The clearance rates will now be used for developing in silico OP metabolism models.

3110 Carboxylesterase Inactivation by Chlorpyrifos: Immunotoxic or Protective in the Murine Lung?
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Carboxylesterases (Ces) are enzymes that metabolize xenobiotics and anti-inflammatory lipid mediators. We had previously observed that female Ces1d$^{-/-}$ mice exhibited a heightened immune response to LPS compared to their wild type (WT) counterparts with respect to Il6 mRNA levels in lung. Chlorpyrifos (CPF) is an organophosphate (OP) insecticide that inhibits Ces activity in vivo at doses that do not inhibit acetylcholinesterase, the canonical target of OPs. Previous data from our lab demonstrated that chronic low-dose exposure to CPF could inactivate Ces in the mouse lung. In our initial immune studies, we discovered that pretreatment of neonate and adult mice with CPF did not alter LPS-induced Il6 mRNA levels in WT mice (p<0.05, two-way ANOVA). In addition, no significant CPF-mediated changes in LPS-induced Il6 and Il1b mRNA levels in either mouse strain were found. Our preliminary results support the hypothesis that Ces protects against adverse immune effects in murine lung caused by CPF exposure; additional studies are needed to confirm these results and to further investigate the protective effects of Ces on lung health. Supported by NIH R1S GM128206.

3111 Adipocyte Differentiation and/or Lipid Accumulation Are Induced by Commonly Used Pesticides in Indiana
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Obesity and obesity-related disorders remain a threat to personal and public health - a trend that is apparent in Indiana wherein nearly 34% of rural residents are obese and symptomatic with related cardio-metabolic disorders. Factors related to excessive fat accretion are multifaceted including genetics, caloric intake, and physical inactivity, however; mounting evidence suggests environmental factors, such as exposure to chemical pollutants, can also contribute to obesity-related diseases. Here, we sought to investigate whether commonly used agricultural pesticides used in corn and soy fields can enhance adipocyte differentiation and lipid droplet formation in vitro. We treated the mouse 3T3-L1 preadipocyte cell line with increasing concentrations of atrazine, flumetsulam, acetochlor, metolachlor, dicamba, tebufentin, or glyphosate followed by staining for lipids. Of these chemicals, exposure to acetochlor, metolachlor, tebufentin and atrazine increased lipid staining. Through qPCR we determined that these four chemicals also increased the expression of Fabp4, PPARg, Lpl and Fsp27, which are markers for pre-adipocytes (early differentiation), differentiating adipocytes, mature adipocytes, and lipid droplet respectively. Future studies will determine if these and other chemicals will affect lipid regulation in flies, fish and mice, with the long-term goal of providing the Indiana rural community with information on how to best avoid exposure to obesogenic chemicals.

3112 Multi-omics Phenotyping of the Gut-Liver Axis Allows Health Risk Predictability from Subchronic Toxicity Tests of a Low-Dose Pesticide Mixture in Sprague-Dawley Rats

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Health effects of pesticides, as mixtures, are not always accurately detected using the current battery of regulatory toxicity tests. We compared standard histopathology and serum biochemistry measures and multi-omics analyses in an in vivo subchronic toxicity test of a mixture of six pesticide active ingredients frequently detected in foodstuffs (azoxystrobin, boscalid, chlorpyrifos, glyphosate, imidacloprid and thiabendazole) in Sprague-Dawley rats. Analysis of water and feed consumption, body weight, histopathology and serum biochemistry showed little or no physiological effects. Contrastingly, serum and caecum metabolomics revealed that nicotineamide and tryptophan metabolism were affected, which suggested the activation of a cell danger response, which could include oxidative stress. Glycerophospholipids, serotonin, pyridoxal and nicotinamide riboside accumulated in the caecum. This was not reflected by gut microbial community composition changes evaluated by shotgun metagenomics. Transcriptomics showed that 257 genes had their expression changed. Gene functions affected included the regulation of response to steroid hormones and the activation of stress response pathways. Genome-wide DNA methylation analysis of the same liver samples showed that 4255 CpG sites were differentially methylated (> 10% difference). We built OPLS-DA models to assess the predictive ability of the different omics approaches used in this study. Serum metabolomics (pR2Y = 0.003, pQ2 = 0.001), liver transcriptomics (pR2Y = 0.10, pQ2 = 0.002), and to a lesser extent the caecum metabolomics (pR2Y = 0.16, pQ2 = 0.003), discriminated the pesticide-treated group from the concurrent control group. Shoigu metabonomics in caecum and genome-wide methylation in liver did not significantly discriminate between the two experimental groups. Overall, we demonstrated that unlike standard blood biochemical and organ histological analysis, in-depth molecular profiling in laboratory animals exposed to low concentrations of pesticides reveals metabolic effects on the gut-liver axis, which can potentially be used as biomarkers for the prediction of future negative health outcomes. Our data suggest that adoption of multi-omics as part of regulatory risk assessment procedures will result in more accurate outcome measures, with positive public health implications.

3113 Using Small Molecule Tool Inhibitors for Early Target Safety Assessment in Drug Discovery: VPS34 Case Study


Vps34 is a master regulator of endosome maturation and autophagosome biogenesis. In addition, VPS34 is highly ligandable for designing potent and selective small molecule inhibitors. Thus, VPS34 has been an attractive drug target with the steep dose-to-target toxicity response of these tool compounds, our oral VPS34 inhibitor program as a drug target was terminated. In conclusion, the present study utilized tool molecules to enable an early no-go decision and helped prioritization of drug discovery efforts to tissue-targeted delivery or inhibition of autophagy-specific pathway components to drive anti-tumor immunity.
To answer the growing demand for a fast, reliable and cost-effective methodology to detect the chemical substances toxicity (particularly new drugs and medications in Human Risk Assessment), several laboratories across the world are joining forces to develop, standardize, harmonize and promote Zebrafish as a new test method. In this scenario, we are acting on several fronts: participating in a large number of global and multi-laboratories initiatives but also boosting our internal R&D in order to validate it as preclinical alternative model and achieve soon the approval of international test guidelines.

In the project presented here, we exposed zebrafish embryos from 0 to 96 h post fertilization to a battery of 31 compounds classified as teratogens or non-teratogens in mammals to prove its predictivity. The teratogenicity score was based on the morphometric analysis of 16 phenotypical parameters, namely heart edema, pigmentation, body length, eye size, yolk size, yolk sac edema, otic vesicle defects, otolith defects, body axis defects, developmental delay, tail bending, scoliosis, lateral fins absence, hatching ratio, lower jaw malformations and tissue necrosis, using a high throughput automated software. Among the 31 compounds, 20 were detected as teratogens and 11 as non-teratogens, resulting in 94.44% sensitivity, 90.91% specificity and 87.10% accuracy compared to rodents. These percentages decreased slightly when referred to humans, with 87.50% sensitivity, 81.82% specificity and 74.19% accuracy, but allowed an increase in the prediction levels reported by rodents for the same compounds. Positive compounds showed a high correlation among all teratogenic phenotypes, pointing out at general developmental delay as major cause to explain the physiological/morphological malformations. A subsequent analysis based on deviations from main trends revealed potential specific modes of action for some compounds such as retinoic acid, DEAB, octrhodrin, valproate, valproic acid, acetyaminophen, dassatinib, imatinib, methamethoxan, 6-aminoantinamide and bisphenol A. The high degree of predictivity and the possibility of applying mechanistic approaches makes zebrafish a powerful model for screening teratogenicity.

Cathespin K (CatK) is a major lysosomal protease and a promising osteoporosis drug target due to its role in degrading bone organic matrix, known as bone resorption. It exerts its function when released from active osteoclasts, thus inhibiting CatK is suggested to decrease bone resorption, without decreasing bone formation. However, besides its high expression in bones, CatK is also found in a variety of other tissues, where CatK inhibition can lead to unwanted effects. Odanacatib developed by Merck is the only CatK inhibitor candidate that so far demonstrated high therapeutic efficacy in patients with postmenopausal osteoporosis in Phase III clinical trial, but it was terminated due to the cardio-cerebrovascular adverse effects. To obtain a broad perspective on the liabilities of CatK inhibition we conducted a meta-analysis of current knowledge. The unique ability of CatK inhibition to dissociate the inhibition of bone resorption from the coupling of T-cells in response to T-cell receptor activation, making it an attractive target for autoimmune diseases such as rheumatoid arthritis. Pyrimidine synthesis is a critical function in embryofetal development, and marketed drugs against another target in this pathway, dihydroorotate dehydrogenase (DHODH), were teratogenic in animal studies. It is proposed however that the expression of a second CTP synthase (CTPS2) and/or the availability of an alternative salvage pathway in the developing embryo could compensate for the loss of CTPS1 activity and avoid the potential adverse effect of developmental toxicity. In humans, deficiency in CTPS1 leads to profound immunodeficiency, but no other clinical symptoms. We investigated the potential of in vitro assays from a number of providers, using human induced pluripotent stem cells (iPSC) or zebrafish larvae to rapidly and cost-effectively evaluate the developmental toxicity of compounds inhibiting pyrimidine synthesis. While hiPSC cell assays were found to be unsuitable for monitoring the developmental toxicity of inhibitors of this pathway due to unusual dose responses in the metabolic parameters measured, assays in developing zebrafish larvae were able to clearly demonstrate the toxicity of DHODH inhibitors. Prototype CTPS1 inhibitor compounds, synthesized based on published structures and with demonstrated differential activity against CTPS1 and CTPS2 in vitro, showed greatly reduced developmental toxicity in the zebrafish assay compared to DHODH inhibitors, derepressing their potential for a more favorable safety profile. The zebrafish assay offers an inexpensive and rapid tool to screen and rank pyrimidine biosynthesis inhibitors for developmental toxicity potential.

Cereblon E3 ligase modulator drugs (CELMoDs) bind to human cereblon to recruit selected substrates of the CRL4(Cub) E3 ubiquitin ligase for degradation. Pharmacologic degradation of the translation termination factor G1 to S phase transition (GTP1) results in anti-tumor activity. Dose dependent hypocalcemia has been observed with the test article CC0781325, a potent oral GSP1-1 degrader. Binding of CELMoDs to rodent CRBN does not result in G1 degradation, but human cerbLon knock-in (huCRBN KI) mice, a transgenic mouse model engineered to express human cereblon that is capable of recruiting and degrading substrates including GSP1, showed greatly reduced developmental toxicity in the zebrafish model and achieve soon the approval of international test guidelines. In the project presented here, we exposed zebrafish embryos from 0 to 96 h post fertilization to NPS 2143 could not reverse the decreases in serum iCa2+ and PTH caused by CC0781325. Increases in serum iCa2+(26%) and PTH (375%) were observed in Group 3. The exposure of NPS 2143 was 6 and 16 µM.hr in Groups 3 and 4, respectively, with the T_max of 2-4 hours. Relative to the T_max of 2 hours. The exposure of NPS 2143 was 6 and 16 µM.hr in Groups 3 and 4, respectively, with the T_max of 2-4 hours. Relative to corresponding Group 1 time points, significant decreases in serum iCa2+ (35%) and PTH (88%) were observed in Group 2 at 4-hr postdose, while significant increases in serum iCa2+ (26%) and PTH (375%) were observed in Group 1. The mice treated with CC0781325 + NPS 2143 had significant decreases in serum iCa2+ (27%) and PTH (78%). These results demonstrated that treatment with NPS 2143 could not reverse the decreases in serum Ca2+ and PTH caused by CC0781325, confirming that CC0781325 prevented the production of PTH resulting from decreased PTH synthesis caused by reduced PTH mRNA in parathyroid gland of CC0781325 treated mice when compared to vehicle or NPS 2143 treated mice. Our data showed that hypocalcemia caused by CC0781325 was due to decrease in PTH resulting from decreased PTH synthesis caused by reduced PTH mRNA in parathyroid gland. Possible mechanisms for this effect to be investigated include reduced transcription or altered stability of PTH mRNA.
The association between diabetes associated hypertension and reproductive disorder has long been a matter of debate. It is therefore noteworthy to know whether testicular impairment is the result of diabetic per se, or an adverse effect of antihypertensive treatment, or a combination of both. Among the most used antihypertensive drugs, the beta (β)-blockers have found to be beneficial therapeutically in treating hypertensive diabetic patients. It has been accounted that antihypertensive regimens in diabetic individuals severely influence the testicular dysfunction. Present study was aimed to compare the effect of classical β-blocker atenolol with third generation beta (β)-blocker nebivolol in diabetic rat testes. To elucidate the role of these β-blocker in diabetic testes, Sprague Dawley rats were randomized into 8 groups, control group, animal receiving nebivolol or atenolol served as atenolol/nebivolol (10 mg/kg) group, diabetic group and diabetic animal receiving atenolol (10 mg/kg) and nebivolol (2.5, 5 and 10 mg/kg). Diabetes was induced by single dose (55mg/kg, i.p.) of streptozotocin (STZ). After the 4 weeks treatment, the animals were sacrificed and blood glucose level, sperm counts, sperm head abnormality, sperm DNA damage, histology, immunohistochemistry of 8-oxy-dG and western blotting of NF-xB, COX-2, caspase-3, p-38 and e-NOS were performed. Results showed that nebivolol but not atenolol significantly decreased the sperm counts, sperm DNA damage and reduced the sperm head abnormality in diabetic rat. Further, nebivolol significantly reduced the number of 8-oxy-dG cells that showed it decreased the oxidative DNA damage. Western blotting results showed that caspase-3, COX-2 and NF-xB expression were significantly down-regulated by nebivolol while atenolol leads to insignificant changes in diabetic testes. In conclusion, we propose that nebivolol may be considered as a potential candidate for treatment in diabetic patients who are prone to testicular damage.

Development of Rat Models of Acute Respiratory Distress Syndrome (ARDS) for Efficacy and Safety Assessment


Acute Respiratory Distress Syndrome (ARDS) is a life threatening condition associated with pneumonia, sepsis and trauma; the mainstay of treatment is to support lung function. Recent data suggest there are subpopulations of ARDS patients; therefore improved understanding of the mechanisms that lead to lung injury will help to target therapies (Carla et al, Respir Res 21, 81, 2020). The objective of this study was to develop rat models of experimental ARDS using clinically relevant, but distinct challenges, to compare acute lung injury associated with pneumonia, sepsis and trauma; the mainstay of treatment is to support lung function.

Salbutamol is known to possess selective to β2-adrenoceptors agonistic action. Thus, it is imperative to investigate the potential therapies for muscle wasting associated with diabetes. In recent years, stimulation of β2-adrenoceptors has fascinated the interest of clinicians for ameliorating the condition associated with diabetes. In recent years, stimulation of β2-adrenoceptors has fascinated the interest of clinicians for ameliorating the condition associated with diabetes. In recent years, stimulation of β2-adrenoceptors has fascinated the interest of clinicians for ameliorating the condition associated with diabetes. In recent years, stimulation of β2-adrenoceptors has fascinated the interest of clinicians for ameliorating the condition associated with diabetes.

Ameliorative Effect of Salbutamol in Diabetes-Induced Skeletal Muscle Loss

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India is one of the epicenters of diabetes and has the second-highest diabetic population in the world after China. Recent studies showed that diabetes accelerates muscle wasting and worsened the condition of skeletal muscle. Thus, it is imperative to investigate the potential therapies for muscle wasting associated with diabetes. In recent years, stimulation of β2-adrenoceptors has fascinated the interest of clinicians for ameliorating the condition associated with diabetes. In recent years, stimulation of β2-adrenoceptors has fascinated the interest of clinicians for ameliorating the condition associated with diabetes. In recent years, stimulation of β2-adrenoceptors has fascinated the interest of clinicians for ameliorating the condition associated with diabetes. In recent years, stimulation of β2-adrenoceptors has fascinated the interest of clinicians for ameliorating the condition associated with diabetes.
significantly restored the inflammatory markers in serum, restored abnormal antioxidative markers, and muscle architecture in diabetic skeletal muscle. Overall, salbutamol could be used as a potential drug to treat skeletal muscle loss in STZ-induced diabetes.

3123 Potential Hepatoprotective and Antifibrotic Effect of Piperine in Carbon Tetrachloride-Induced Liver Fibrosis in Wistar Rats


Hepatic fibrosis is a prime cause of mortality that ultimately leads to cirrhosis. Liver fibrosis is a dynamic and reversible process that is characterized by an imbalance between the formation and degradation of extracellular matrix (ECM) which is rich in fibrillar collagens. Long pepper, known as pippali, is the most widely used medicinal herb in the traditional Indian medicine system. It's medicinal properties are mainly due to the action of piperine. Piperine is an alkaloid present in seeds of black pepper (Piper nigrum) and long pepper (Piper longum) belongs to family Piperaceae. Present study was aimed to investigate the effect of piperine in CCl₄-induced liver fibrosis in rat. For the experimental study, male Wistar rats (n=6) were randomized into four groups. Group I received vehicle and served as control. Group II received three doses of CCl₄ per week (100 μl/g) for consecutive six weeks and served as a fibrosis group. Group III received CCl₄ and Valsartan (25 mg/kg) and Group IV received CCl₄ and Piperine (100 mg/kg) per week (100 μl/g) for consecutive six weeks. Further, the doses of piperine and valsartan were given for consecutive six weeks after the induction of fibrosis. Result showed that piperine treatment significantly restored the liver function biomarkers (ALT, AST & bilirubin) and oxidative stress parameters (MDA, GSH and SOD). Further, piperine significantly reduced the percentage fibrotic area as shown by Masson’s trichrome staining and improved the cellular structure of liver as evaluated by histology. In conclusion, piperine improved liver enzyme status and ameliorated the carbon tetrachloride-induced rat liver fibrosis. Piperine could be used as an effective therapeutic candidate against CCl₄-induced liver fibrosis. Further, detailed molecular studies are required to elucidate the protective role of piperine in CCl₄-induced liver fibrosis.

3124 Parental Cadmium Exposure Increased Hepatocellular Carcinoma Risk of the Male Offspring

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Cadmium (Cd) is often taken up by inhaling contaminated smoke and from ingestion of food grown in contaminated soil. Accumulation of Cd in the organs, such as the liver and kidney, results in the malignant transformation of cells via complex mechanisms. Therefore, Cd is classified as a human carcinogen by the International Agency for Research on Cancer. In our previous study, we treated rats and mice with low doses of Cd for 4 weeks and found that TNF-α-initiated caspase-8 dependent apoptotic signaling pathway is suppressed and oxidative DNA damage was increased, suggesting the potential risk of carcinogenesis. Considered that Cd exposure of parents may increase their offspring’s susceptibility to liver cancer, we examined whether parental exposure to low-dose Cd (5 ppm) increased hepatocellular carcinoma (HCC) risk in their male offspring. Immediately after weaning, hepatic Cd levels were significantly increased and global DNA methylation levels were significantly decreased in the offspring of parents exposed to Cd compared to those of parents without Cd exposure. Also immediately after weaning, male offspring of parents exposed to 5 ppm Cd through drinking water were given HCC food (high-fat choline-deficient diet / diethylnitrosamine [DEN]) for 26 weeks to induce HCC. Male offspring of parents without Cd exposure were randomly divided into two groups with and without HCC food as negative and positive controls. The experimental results showed that at 26 weeks after weaning, there was no difference in hepatic Cd levels among the three groups. Bodyweight was increased in the offspring of parents exposed to Cd compared to those without Cd exposure. However, parental exposure to Cd increased liver weight, tumor number and tumor size in offspring compared to those fed HCC food but without parental Cd exposure. These data suggested that parental Cd exposure increased susceptibility of male offspring to HCC food-induced liver cancer. Supported in part by USA-China exchange program. JLY is the recipient of NIH grant T32-ES051156.

3125 Circadian Rhythms Modulate House Dust Mite-Induced Lung Immune-Inflammatory Response and Airway Remodeling in Mice

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House dust mite (HDM) exposure induces lung inflammation and airway remodeling characterized by an accumulation of immune-inflammatory cells, increased airway hyperresponsiveness and mucus production. Sex differences exist in the circadian timing system, but their exact role in the time-of-day effect of HDM-induced altered immune response during asthma remains elusive. We hypothesize that HDM exposure differentially impacts immune-inflammation response, likely due to a sex-dependent difference in asthma associated with circadian disruption. C57BL6/J mice (8 weeks old; females and males) were exposed to HDM (30 µg) or control (PBS) via intranasal route for 10d at Zeitgeber Time [ZT0: 6 am or ZT12: 6 pm]. Lung tissues and blood were collected 48 hours post-last exposure. Differential cells in the lungs, serum IgE, corticosterone levels and transcripts of core circadian clock-controlled genes (CCGs) were analyzed. Immunophenotyping analysis revealed time (ZT0 vs. ZT12) and sex-based (females vs. males) difference in immune-inflammatory response following HDM exposure. Lung resident eosinophils (rEos) and interstitial macrophages (iMac) were significantly increased in the HDM-exposed group at ZT0 and ZT12 compared to controls. Sex-based comparison revealed a significant increase in rEos among the HDM exposed females compared to males at ZT12 vs. ZT0, PMNs and DCs remain unaltered in HDM exposed females at ZT0 and ZT12, but males showed a significant decrease in PMNs and DCs at ZT12 compared to controls. AMs were decreased in HDM exposed females and males at ZT0 and ZT12. Immunogold markers and dysregulation of CCGs in HDM exposed females vs. males at ZT12. Understanding the role of circadian clock-dependent time-of-day response and gender differences will aid in developing therapies for asthma. Supported by the NIH R01 HL142543 (IKS).

3126 Enhancing the Environment for Rodents in Toxicology Studies


Animal welfare is a priority in the conduct of toxicology studies and evidence-based animal welfare research offers an approach to quantify the interest and the impact of selected enrichment and structural components. 10 Rattus norvegicus, (Male and female Sprague-Dawley rats, 200-380 grams), were divided in groups (2 animals per bin) with different enrichments (i.e., ladder, hideout on cage floor, suspended hideout, running wheel, metal basket, and a suspended tube) and each group was evaluated in the presence of each enrichment item for a period of three days. CD1 mice were allocated to trios in bins and the use of the running wheel was quantified. Within cage activities were recorded by camera during the experimentation period. The rats were monitored for 18 days (3 days for each enrichment device) and mice for 3 days. The frequency and the duration of utilization per day was quantified and analyzed. The enrichment devices for rats showed different mean daily duration and mean frequency of use: running wheel (1h00/day; 47 times/day), ladder (4h52/day; 193 times/day), hideout on the cage floor (6h51/day; 88 times/day), suspended hideout (10h38/day; 108 times/day), suspended tube (12h22/day; 128 times/day) and a metal basket (1h430/day; 123 times/day). For mice, the mean duration was evaluated over 3 days. Our data showed that the mice used the running wheel for an average of 5h28/day/bin. The results presented herein suggest a higher interest for resting and hiding structures that were elevated in rat cages while the ladder was the device most frequently used by this species. In contrast, the time spent in the running wheel was limited for rats. In mice, the running wheel occupied a considerable proportion of the daily activities suggesting a high interest for this enrichment device.
3127 Hindlimb Unloading-Induced Cellular Toxicity and Oxidative Stress in the Skeletal Muscle and Colon of Rats: Linking Gut-Skeletal Muscle Axis


The inability of physical functions due to skeletal muscle loss or mechanical unloading is mainly due to immobilization, prolonged bed rest, and persistent inactivity. The hindlimb unloading (HU) closely resembled the responses to spaceflight and may lead to a significant loss of skeletal muscle strength which eventually causes muscle atrophy. It is widely used to explore the musculoskeletal system to investigate muscle atrophy. The objective of the present study was to appraise the cellular toxicity and oxidative stress in gastrocnemius muscle and colon of rat in the hindlimb unloading model. For the experiment, male Wistar rats were used and randomized into two groups. In one group, hindlimb-unloaded rats were suspended via the tail with no load-bearing on hindlimbs and termed as HU group, another group of animals were without load bearing and serve as a control. After 14 days, rats were sacrificed and gastrocnemius muscle and colon were dissected and used for estimation of protein content, oxidative stress, and cellular toxicity. Results showed that HU group showed a significant reduction in body weight, gastrocnemius muscle, and colon weight when compared to the control group. Next, we measured the protein content in the gastrocnemius (GN) muscle and colon. Our results showed that there were significant reductions in protein content in HU group as compared to the control. Further, malondialdehyde (MDA) levels were measured and found to be increased in both GN muscle as well as in the colon of HU model. Next, we estimate antioxidant statuses such as reduced glutathione (GSH) and superoxide dismutase (SOD) levels that were markedly decreased in both tissues of HU group as compared to the control. A histopathological study also showed a decrease in minimum Feret’s diameter and cross-sectional area of GN muscle; crypt length and crypt number in the colon of HU when compared to control. Findings of the present work indicate that hindlimb unloading not only deteriorated the skeletal muscles of the hindlimb but also affect the colon environment as evident from significant changes in oxidative stress, antioxidant defense system, and cellular structure of gastrocnemius skeletal muscle and colon. Further research in this field is needed to better explain the skeletal muscle wasting that could associate that disruption of intestinal homeostasis is due to the skeletal-muscle loss.

3128 Docosahexaenoic Acid (DHA) Suppresses Broad Spectrum of Inflammatory Proteins Elicted in a Murine Model of Silica-Triggered Lupus Flaring

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Intranasal instillation of lupus-prone mice with crystalline silica (cSiO2), a respirable occupational toxicant linked to human autoimmune disease, mimics lupus flaring in humans by accelerating systemic autoimmunity and glomerulonephritis. Notably, the inflammatory effects were blunted when mice were supplemented with physiologically relevant levels of DHA, an omega-3 polyunsaturated fatty acid (ω-3) found in fish oil. Here we tested the hypothesis that DHA suppresses cSiO2-induced inflammatory proteins (IPs) overall time in this model. Female NZBWF1 mice, at 6 weeks (wks) of age, were fed isocaloric diets of AIN-93G supplemented with a DHA 0, 4, or 10 g/kg diet beginning 2 wks before cSiO2 instillation. Mice were intranasally instilled with 1 mg cSiO2 or vehicle alone once per wk for 4 wks, starting at 8 wks of age. Cohorts were sacrificed 1, 5, 9, or 13 wks after the last cSiO2 installation. Bronchoalveolar lavage fluid (BALF) was subjected to High-throughput multiplex ELISA array profiling for 203 IPs. At 9 and 13 wk, cSiO2-induced 103 and 102 IPs, respectively (p <0.05) in mice fed with control diet. IPs that were induced included chemokines, adhesion molecules, co-stimulatory molecules, enzymes, signal transduction proteins, growth factors, TNF superfamily proteins, and cytokines. In DHA-supplemented mice, 46 and 37 cSiO2-triggered cSiO2-, induced IPs were significantly inhibited at 9 and 13 wk, respectively (p <0.05). Most notably for chemokines, cSiO2-induced IPs and inhibition by DHA strongly correlated with gene expression, pulmonary ectopic lymphoid structure development, and autoantibody production. IP profiles showed a negative correlation with c-SiO3 index, an erythrocyte biomarker of c-SiO3 content in tissue phase images. Bioinformatic pathway enrichment using QIAGEN Ingenuity Pathway Analysis tool revealed that inhibitory effects of DHA were associated with inhibition of ARE-mediated mRNA decay, TREM1 signaling, NF-KB signaling, and STAT3 pathways and with activation of PPARα/RXRα and LXR/RXR pathways. Our findings provide preclinical evidence that DHA supplementation alone or as an adjunct to current therapeutics have promise preventing environmentally triggered lupus and other autoimmune diseases.

3129 Evaluation of Dermal Sensitization and Genetic Toxicity Induced by Dimethyl Amine Borane

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Dimethyl amine borane (DMAB) is a reducing agent used in the production of circuit boards, semiconductors, and power transistors. Human exposure occurs primarily through dermal and inhalation exposure in an occupational setting. Metabolism and distribution studies demonstrated that DMAB is absorbed moderately well by both rat (23-26%) and human (41%) skin. The NTP conducted an assessment of the toxicity of DMAB following dermal exposure. To evaluate the potential for DMAB to induce dermal sensitization, the test article (5-50% w/v in 4:1 acetone:olive oil (AOO)) or vehicle control (4:1 AOO) was applied to the dorsa of both ears of female Balb/c mice daily for three days in a combined local lymph node (LLNA)/primary irritancy assay. In the mouse ear swelling test (MEST), the mice were sensitized on the back for 3 days, rested for 4 days, and challenged on the right ear. Dermal treatment with 50% DMAB induced a significant, dose-dependent (p <0.05), increase in lymph node cell proliferation (p3. These data suggest that DMAB may induce an inflammatory hypersensitivity. Sex differences in increased IL-10 and IL-5 (57%) with DMAB did not increase ear swelling in the MEST. DMAB induced dermal irritation at >10% (p <0.05). Mutagenicity of DMAB (125-6000 μg/plate) was also evaluated in two independent studies in three strains of bacteria, with and without rat liver S9 mix for metabolic activation. Weak, but reproducible, dose-dependent increases in revertants were seen in the absence of S9 in Salmonella typhimurium strain TA100 (GC DNA target) and in the presence of S9 in Escherichia coli strain WP2 uvrA/pKM101 (AT DNA target); both of these strains mutate via base substitution. No mutagenic activity was observed in the presence or absence of S9 in Salmonella typhimurium strain TA98, a strain that mutates via frameshifting. Collectively, these data indicate that DMAB is genotoxic, may induce irritant and allergic dermatitis, and may pose a health hazard in the occupational setting.

3130 Omega-3 Fatty Acid Docosahexaenoic Acid (DHA) Suppresses Proinflammatory Cytokine/Chemokine Secretion and Cell Death in Alveolar Macrophage-Like MPI Cells


Respiratory exposure to crystalline silica (cSiO2), a respirable occupational toxicant, leads to the development of pulmonary inflammation, which can contribute to the development of lupus and other autoimmune diseases. Alveolar macrophages (AM) phagocytose cSiO2, which induces a vicious cycle of phagolysosomal permeabilization, inflammasome activation, proinflammatory cytokine/chemokine release, and cell death. These actions give rise to unresolved pulmonary inflammation. In genetically susceptible individuals, this can result in the development of systemic autoimmunity. Here we employed Max Planck Institute (MPI) cells, a novel AM model derived from fetal liver, to: 1) assess cSiO2’s effects on cytokine/chemokine secretion and cell death with and without LPS priming and 2) determine how intervention with the omega-3 fatty acid DHA influences cSiO2-, induced cytokine/chemokine secretion and cell death. MPI cells were i) preincubated with 25 μM DHA or Vehicle (Veh) for 24 h, ii) primed with LPS (20 ng/mL) or Veh for 2 h, and iii) exposed to 25 μg/cm2 cSiO2 for 1, 3 and 6 h. ELISAs were performed to quantify cSiO2-induced release of the cytokines IL-1α, IL-1β, IL-6, and TNF-α, and the chemokine MCP-1. Following priming with LPS, cSiO2 elicited robust IL-1α, IL-1β, IL-6, TNF-α, and MCP-1 release and inflammasome activation as reflected by IL-1β release at 3 and 6 h compared to time-matched controls (P <0.01); however, there was minimal release without LPS priming but, higher than time-matched controls. DHA pre-incubation significantly suppressed IL-1α, IL-1β, IL-6, and MCP-1 release by cSiO2 at 6 h (P <0.05). The influence of cSiO2 on cell death was assessed by discemblage assay which was determined using lactate dehydrogenase (LDH) assay. The LDH assay indicated cSiO2 caused cell death around 5% within 1 h of cSiO2 exposure, peaking around 25% at 6 h regardless of LPS priming suggesting the occurrence of both inflammasome-dependent and -independent cell death. For all the treatment groups, preincubation with 25 μM DHA for 24 h prior to cSiO2 treatment inhibited LDH release at 3 and 6 h by 8 and 10%, respectively.
Occupational exposure to crystalline silica (cSiO₂) is epidemiologically linked to lupus and other autoimmune diseases. While cSiO₂’s autoimmune effects in humans can be simulated chronically in lupus-prone NZBWF1 mice by repeated exposure, the immediate and short-term effects of the particle in this autoimmune model are as yet unclear. Here, we tested the hypothesis that a single acute cSiO₂ dose causes early presentation of cellular, histopathological, transcriptional, and cytokine responses, leading to autoimmunity. To achieve this, we treated female MRL+/+ mice with TCE (10 mmol/kg, ip, every fourth day) with/without antioxidant sulforaphane (SFN; 8 mg/kg, ip, every other day) for 30 weeks. TCE exposure led to 25 differentially expressed miRNAs out of which 17 were upregulated and 8 were downregulated. We identified two miRNAs (miRNA 21 and miRNA 690) known to be involved in autoimmune disease that included glomerulonephritis, elevated blood urea nitrogen, increased colonic oxidative stress (MDA-protein adducts) and inflammatory markers (CD14 and IL-1β), and decreased tight junction proteins (ZO-2, occludin and claudin-3). In addition, TCE exposure led to systemic inflammatory response, evidenced by increased IL-6 and IL-12 in the serum. Importantly, significant increases in AIH markers, especially anti-nuclear antibodies (ANA) and anti-smooth muscle antibodies (ASMA) together with increased splenic and hepatic CD4+ T cells were also observed. Our studies thus delineate how imbalance in gut microbiome and mucosal redox status together with mucosal immune system and gut permeability changes could be the key factors in contributing to TCE-mediated AIH. Apart from suggesting a role of gut microbiome dysbiosis in AIH, our findings could also help in designing specific microbial therapeutic modalities.

Trichloroethene (TCE), an occupational and ubiquitous environmental contaminant, is associated with the induction of autoimmune diseases (ADs). Although oxidative stress has been shown to play a major role in TCE-mediated autoimmunity, the underlying molecular mechanisms still need to be delineated. Altered non-coding RNAs, including microRNAs (miRNA) can influence target genes and contribute to ADs, especially by modulating apoptosis, inflammation and autoimmunity-related genes. This study was, therefore, focused on delineating the contribution of miRNAs in TCE-mediated autoimmune response. To achieve this, we treated female MRL+/+ mice with TCE (10 mmol/kg, ip, every fourth day) with/without antioxidant sulforaphane (SFN; 8 mg/kg, ip, every other day) for 6 weeks. Liver miRNA expression profiles were generated using a miRNA microarray. TCE exposure led to 25 differentially expressed miRNAs out of which 17 were upregulated and 8 were downregulated. We identified two miRNAs (miRNA 21 and miRNA 690) known to be involved in inflammation and autoimmunity, which were further validated by RT-PCR. Interestingly, TCE-mediated increases in miRNAs 21 and 690 were ameliorated by SFN supplementation. SFN treatment also resulted in Nrf2 activation and attenuation of TCE-mediated activation of NF-κB, p-38 MAPK and downstream inflammatory cytokines TNF-α and IL-12. Since miRNA-21 is involved in immune cell activation, its role was further evaluated by conducting in vitro studies (RAW 264.7 cells) using antagonim and mimic of miRNA-21, which resulted in the modulation of a dependent gene NFκB. These studies suggest that TCE-mediated aberrant regulation of miRNA could be an important mechanism leading to inflammation and autoimmunity. Furthermore, attenuation of TCE-mediated responses via suppression of miRNAs support that along with these miRNAs, antioxidant SFN could be a potential therapeutic candidate for inflammation and ADs.
Cannabidiol (CBD) is a phytocannabinoid that encompasses a major constituent of extracts isolated from cannabis plants. CBD has garnered widespread attention due to the potential of its therapeutic application in neuroinflammatory and autoimmune disorders. CBD poses as a non-toxic and relatively inexpensive alternative to current market anti-inflammatory medications that are associated with inadvertent side effects and exorbitant costs. Despite growing enthusiasm of CBD as a complementary and alternative medicine, there is still little understanding on how CBD supplementation limits excessive inflammation in the clinical setting. We aimed to study how CBD limits excessive neuroinflammation by administering CBD via oral gavage (20mg/kg) in a murine model of multiple sclerosis known as experimental autoimmune encephalomyelitis (EAE). Our initial results demonstrated that CBD significantly reduces EAE severity by ameliorating the neuroinflammation-related paralysis symptoms by reducing the number of infiltrating macrophages and increasing the number of myeloid derived suppressor cells (MDSCs). In order to better understand how CBD limited macrophage infiltration, we looked at the transcriptome of the immune cell fraction isolated from the CNS of EAE mice using single cell RNA sequencing (scRNAseq). The scRNAseq results indicated that the macrophages infiltrating the CNS of control mice produced high levels of the pro-inflammatory cytokine, interleukin 1 beta (IL-1β) in comparison to the CBD treated mice. We validated these results by confirming the expression of IL-1β transcripts in myeloid cells isolated from the CNS of vehicle treated mice was significantly higher than that of CBD treated mice. We further confirmed that CBD treatment inhibits IL-1β production by using bone marrow derived macrophages (BMDM) treated with lipopolysaccharide (LPS) to induce an inflammatory response. CBD treatment of BMDM under LPS-stimulated conditions resulted in a significant reduction in IL-1β expression as well as no detectable levels of IL-1β being secreted. In summary our results demonstrate that CBD treatment results in an anti-inflammatory shift in the myeloid cells infiltrating the CNS by inhibiting the production of the pro-inflammatory cytokine, IL-1β in a murine model of autoimmune neu- roinflammation.

Expression Changes in Mice

Cyp1b1-Derived Lipid Epoxydes Modulate TRPA1 Activity in the SNL Model of Chronic Pain


Chronic pain lasts beyond the time required to heal an injury, which can severely affect a person’s life. Chronic pain therapies include NSAIDs, opioids, and TNF inhibitors. However, none of these are universally efficacious, or without risks. New therapeutic options are needed. Transient receptor potential (TRP) ion channels regulate inflammation and pain following injury. Understanding how endogenous TRP ligands change during inflammation and the development of chronic pain may provide new insights on how to prevent or treat chronic pain. Transcriptomics and targeted lipidomics were used to determine changes in lipid metabolizing enzymes and lipids in mouse dorsal root ganglia (DRG) neurons following spinal nerve ligation (SNL). Results revealed a robust induction of Cyp1b1 in both injured DRGs and adjacent spinal cord tissue, as well as changes in the CYP-derived lipid epoxydes 8,9-EET and 19,20-EpDPA. We hypothesized that endogenous lipids produced by Cyp1b1 may be involved in the development of chronic pain. Production of 8,9-EET and 19,20-EpDPA by human and mouse CYP1B1 was confirmed in vitro. Also, 8,9-EET and 19,20-EpDPA selectively and dose-dependently affected the activity of human and mouse TRPA1 in over-expressing TRPA1 HEK-293 cells and cultured DRG neurons, serving as both a direct agonist and sensitizer. The effects on TRPA1 were also attenuated by A697079, a TRPA1 inhibitor. In mice, intraplantar injection of 19,20-EpDPA also induced mechanical, but not thermal hyperalgesia, which was blocked by A697079. These data suggest that alterations to TRP signaling following nerve damage may result from the biosynthesis of endogenous CYP-derived epoxydes that are TRPA1 agonists. Efforts are underway to determine the role of CYP1B1 and other related epoxydes in the development of inflammation and chronic pain, which could advance our understanding of specific molecular processes that contribute to pain of CBD. Understanding the mechanism by which CBD reduces inflammation, as well as neuronal pain, may identify potential targets for poten- tionally preventing and/or treating pain. Support: DoD W81XWH-17-1-0413 and GM121648.

Chronic Organic Dust Exposure Induces Lung Inflammation and Cancer-Related Gene Expression Changes in Mice


Exposure to agricultural organic dusts is associated with inflammation-related lung diseases including asthma, bronchitis, and COPD—all risk factors for the development of lung cancer. Prior studies have examined the inflammatory effects that dust extracts (DE) from swine confinement facilities can have on the lung; however, none have yet to do an in-depth assessment of the transcript- and pathway-level changes associated with chronic DE exposure. A/J mice were intranasally challenged with saline or DE three times a week for 24 weeks. At the start of week 4, mice were given a one-time 100 mg/kg intraperitoneal (i.p.) injection of the tobacco-specific carcinogen, NNK, which has been shown to induce lung tumorigenesis. This study investigated whether chronic DE exposure will alter inflammation and/or cancer-related transcript- and pathway-level gene expression in the lung. Twenty-one weeks post NNK injection, mice were euthanized, and left lung tissues were collected, homogenized, and assessed for transcript and pathway-level gene expression changes using the NanoString Mouse Pan Cancer Immunology panel. Sample normalization and data processing was performed using the nSolver Advanced Analysis Program. Through Principle Component Analyses we identified DE exposure (as opposed to NNK) to be the driving factor in gene/pathway expression alterations. Overall, 112 genes were differentially expressed (p < 0.05) among DE vs. saline samples,12 among NNK vs. no NNK samples, and 5 among the no NNK/DE vs. NNK/DE sample set. There was a strong gene signature for several lung cancer-related genes within the NNK vs. no NNK sample set. Additionally, within DE vs. saline samples, we saw decreased expression in CDH1 and increased expression in SNAI1 and TWIST1—all hallmarks of epithelial to mesenchymal transition (EMT). We identified DE exposure was the main driver for a significant (p < 0.0001) up-regulation in gene expression pathways such as innate and adaptive immunity, cancer progression, and inflammation within DE vs. saline samples, regardless of NNK treatment. Conversely, NNK seemed to have a suppressive effect in several pathways involving TLR signaling-, cell cycle-, and TNF superfamily-related genes. The results from this study provide an in-depth assessment of the transcriptomic and pathway-level alterations that chronic DE exposure can have on an exposed individual.
We propose that inhibiting lysosomal BK channel activity with paxilline will reduce influx into the lysosome, leading to LMP and NLRP3 inflammasome activation. BK channel inhibitors, paxilline, 30-minutes prior to cSiO₂ (50 μg/mL) exposure were assessed after cSiO₂ exposure at 2, 4, 6, and 24 hr. Paxilline significantly decreases cSiO₂-induced cell death, LMP, and NLRP3 activity. These findings suggest that cell surface TSPO in circulating leukocytes decreases cSiO₂-induced cell death, lysosomal membrane permeability, measured by digitonin extraction assay; and NLRP3 activity, characterized by IL-1β systemic inflammation. The alveolar macrophage is the primary-responder in innate immune cell to inhaled cSiO₂. Macrophage phagocytosis of cSiO₂ leads to lysosomal membrane permeability (LMP), activating the NLRP3 inflammasome and initiating a chronic cycle of inflammation. Therefore, prevention of LMP could be key to resolution of cSiO₂-induced inflammation. LMP-related NLRP3 activation has been linked to a decrease in cytosolic K⁺. Because of the integral role of lysosomal ion channels in maintaining lysosomal homeostasis and the large concentration gradient of K⁺ across the lysosome membrane, the activity of lysosomal potassium channels in LMP is of significant interest. Here we hypothesize that the lysosomal potassium channel, BK, facilitates K⁺ influx into the lysosome, leading to LMP and NLRP3 inflammasome activation. We propose that inhibiting lysosomal BK channel activity with paclitaxel will reduce cSiO₂-induced cell death, LMP, and NLRP3 activity. To test this hypothesis, murine bone marrow-derived macrophages (BMDMs) were pre-treated with BK channel inhibitor, paclitaxel, 30-minutes prior to cSiO₂ (50 μg/mL) exposure (up to 24-hours) with LPS-priming (1ng/mL). Cytotoxicity, LMP, NLRP3 activity were assessed after cSiO₂ exposure at 2, 4, 6, and 24 hr. Paclitaxel significantly decreased cSiO₂-induced cell death, as measured by LDH release; LMP, measured by digitonin extraction assay; and NLRP3 activity, characterized by IL-1β and IL-18 cytokine release. In conclusion, inhibiting lysosomal BK channel activity decreases cSiO₂-induced cell death, lysosomal membrane permeability, and NLRP3 inflammasome activation. Therefore, BK channel activation may be a key regulatory event in cSiO₂-induced chronic inflammation. Funding was provided by R01 ES027353 and P30 GM103338.

**Surface Translocator Protein 18 kDa (TSPO) Localization on Immune Cells upon Stimulation with Lipopolysaccharide and in ART-Treated HIV+ Subjects**

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Translocator Protein 18 kDa (TSPO) is a well-known outer mitochondrial membrane protein and it is widely used as a biomarker of neuroinflammation and brain injury. While it is thought that TSPO plays key roles in a multitude of host cell functions, including steroid biosynthesis, apoptosis, generation of reactive oxygen species, and proliferation, some of these functions have recently been questioned. Here, we report the unexpected finding that circulating immune cells differentially express basal levels of TSPO on their cell surface, with a high percentage of monocytes and neutrophils expressing cell surface TSPO. In vitro stimulation of monocytes with lipopolysaccharide (LPS) significantly increases the frequency of cells with surface TSPO expression in the absence of altered gene expression. Importantly, the LPS-increase in TSPO cell surface expression in monocytes appears to be selective for LPS since two other distinct monocyte activators failed to increase the frequency of cells with surface TSPO. Finally, when we quantified immune cell TSPO surface expression in antiretroviral-treated (ART) HIV+ donors, a chronic inflammatory disease, we found significant increases in the frequency of TSPO surface localization, which could be pharmacologically suppressed with Δ⁶-tetrahydrocannabinol (THC). These findings suggest that cell surface TSPO in circulating leukocytes could serve as a peripheral blood-based biomarker of inflammation.

**E-cigarette Use, Systemic Inflammation, and Depression**

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E-cigarette (e-cig) use (vaping) is an emerging public health problem. Depression has been found to be associated with increased e-cigarette use and vaping and depression are each independently associated with systemic inflammation elevation. To date, the role of inflammation in the relationship between vaping and depression has not been explored. The goal of this analysis was to investigate whether the likelihood of depression among current e-cig users is independently associated with systemic inflammation. Data from the 2015-2016 and 2017-2018 National Health and Nutrition Survey cycles were used (N = 4,961). Inflammation was defined as serum C-Reactive Protein (CRP) ≥ 8.0 mg/L. Depressed individuals were characterized as scoring > 10 on the Patient Health Questionnaire-9 (PHQ-9). Current e-cig users were defined as individuals who reported vaping at least once in the past 30 days (N=644) and were further categorized as exclusive users, dual users (both e-cig and combustible cigarette users) and e-cig users who were previous smokers. The reference group consisted of individuals who were nonsmokers and reported never using e-cigs (N=4,518). Weighted logistic regression analysis adjusting for sex, body mass index, and poverty status was used to determine the odds ratios (OR) for depression among current e-cig users compared to non-users was 3.78 (95% Confidence Interval (CI): 2.59, 5.52). In adjusted analyses the following ORs for depression were found: all current e-cig users with CRP < 8.0 = 3.57 (95% CI: 2.06, 5.51); and CRP ≥ 8.0 = 4.60 (2.48, 8.11); exclusive e-cig users with CRP < 8.0 = 1.91 (95% CI 0.78, 4.69) vs. with those with CRP ≥ 8.0 = 5.09 (1.44, 18.02); and dual users with CRP < 8.0 = 4.31 (2.35, 7.89) and those with CRP ≥ 8.0 = 7.37 (1.85, 29.41). This is the first study that we are aware of to demonstrate that depression among current e-cig users is independently associated with serologic evidence of systemic inflammation.

**Role of Hyperglycaemia in Ulcerative Colitis: Studies on Male and Female BALB/c Mice**


Ulcerative colitis is a subset of inflammatory bowel disease associated with several comorbidities and extra-intestinal manifestations. Diabetes mellitus is the third most frequent comorbidity associated with ulcerative colitis with epidemiological, pathogenetic, clinical and therapeutic implications. Chronic colitis associated hyperglycaemia makes the medical treatment challenging due to difficult control in glucose levels and for high risk post-operative complications. Present study was designed to corroborate the influence of hyperglycaemia as a susceptibility factor to colon inflammation and to gain insight into the molecular mechanisms involved in ulcerative colitis as well as comorbid condition of diabetes mellitus. Both male and female BALB/c mice (8 week old males) were first divided into three groups: Control (CON) provided with normal drinking water; Diabetes (DB) group administered only with Streptozotocin; Ulcerative colitis group administered with 2.5%/w/v Dextrose sulphate sodium (DSS); DSS+DB group (2.5%/w/v DSS + Streptozotocin). To mimic the human pathological condition, dextran sulphate sodium (2.5%/w/v) dissolved in drinking water was given for 3 cycles with 7 days recovery period in between to both male and female BALB/c mice. At the 1st recovery period, Streptozotocin (40mg/kg; i.p.) was administered for 5 consecutive days in case of male BALB/c mice; whereas same procedure was repeated at each recovery period in case of Female animals due to resistant in diabetic model development. In DSS events of male animals, disease activity index, myeloperoxidase activity, nitrite levels, plasma lipopolysaccharides, IL-1β, histological score, % fibrotic area, % goblet area, % TUNEL positive cells were significantly increased. Further, protein expression of NFκB65, PCNA, IL-6, ASC, Caspase-1 significantly increased and catalase level decreased in DSS only group of male animals, whereas milder responses were observed in DSS+DB group of female animals as compared to male animals. The present study findings indicate that hyperglycaemia acts as key driver that deteriorate the pathological conditions of ulcerative colitis in male animals; whereas milder conditions were developed in female animals due to protective effect of estrogens.

**Lipid Raft-Mediated Regulation of NADPH Oxidase: Vaping-Induced Inflammation**

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A recent outbreak of vaping-related illnesses and deaths across the US has sparked an outcry on the use of e-cigarettes. These events have resulted in several restrictions on the sale of e-cigarettes (e-cigs) in recent times. Earlier reports have shown that the molecular consequences of vaping are quite similar to that of conventional smoking. Dysregulated activation of NADPH oxidase and increased localization of its subunits in lipid raft entities has been reported in response to a wide array of stimulants. Our studies using cigarr-ette smoke extract challenged lung epithelial cells showed the important role of lipid rafts in regulating inflammatory responses. In light of these facts, we hypothesized the important role of lipid rafts in regulating the NADPH oxidase.
Food additives, such as emulsifiers, have been reported to induce adverse changes in the microbiome and exacerbate inflammation of the gastrointestinal tract, leading to inflammatory bowel disease (IBD). These induced changes can combine with genetic and environmental factors that are thought to be critical causes of IBD. The mechanism and prevalence of IBD and increased inflammation from damages to the mucosal lining of the GI tract, lead to increased susceptibility to bacterial invasions and a weakened immune system. Inflammation from damages to the mucosal lining of the GI tract, lead to the subcellular localization of its subunits. We are using the strategy to disrupt rafts by methyl-β-cyclodextrin and filipin or altering the composition of raft entities in A549 cells. Overall, our findings demonstrate that the critical role of lipid rafts EVC-induced-NADPH oxidase mediated inflammation and provide a foundation for future translational studies.

**3144 Cytochrome P450 (CYP) 2C8-Derived Linoleic Acid Metabolites Modulate the Inflammatory Response of Human Lung Epithelial Cells**


Asthma causes chronic airway inflammation and bronchial hyper-reactivity. Despite treatment, many patients experience suboptimal symptom control, in part, due to variability in genes that dictate drug disposition. A total of 170 SNPs in genes associated with asthma, and variables of unknown significance, were assessed for effects on asthma control in a pharmacogenetic study. The presence of one or more copies of the cytochrome P450 (CYP) 2C8*3 (R139K/K399R) allele correlated with improved asthma control: Mean asthma control scores were 3.5 (n=184) vs. 4.4 (n=909) for the CYP2C8*1/*1 genotype (p=0.0006). Furthermore, when data were stratified by treatment of corticosteroids, patients with the CYP2C8*3 allele achieved a mean asthma control score of 3.66 (n=89) vs. 5.26 (n=418) for the CYP2C8*1/*1 genotype (p=0.0002). CYP2C8 metabolizes endogenous long-chain polyunsaturated fatty acids. Oxilipin profiling demonstrated reduced concentrations of the linoleic acid-derived metabolite 9(10)-EpOME and its DiHOME metabolite released by human lung epithelial cells engineered to over-express CYP2C8*3 when compared to cells expressing equivalent levels of CYP2C8*1. Further, expression of CYP2C8*3 reduced basal and TNFα-stimulated mRNA levels of IL-6 and IL-8, which are key pro-inflammatory cytokines in severe asthma exacerbations, whereas treatment of normal human lung epithelial cells from both the proximal and distal airways with 9(10)-EpOME alone induced the expression of IL-6, IL-8, IL-1A, and IL-1B mRNA. In this study, roles for the human transient receptor potential ankyrin-1 (TRPA1) and vanilloid-1 (TRPV1) channels in mediating the effects of the linoleic acid-derived epoxides on human lung epithelial cells were found, including previously reported effects on mitochondrial function. 9(10)-EpOME was found to activate both human TRPA1 and TRPV1 channels, and pharmacological inhibition of both channels was found to modulate the aforementioned cellular responses to 9(10)-EpOME. TRP channels are involved in airway diseases, including asthma. These data suggest a critical role for bioactive lipids and TRP channels in inflammation and future studies should further our long-term goal of improving the treatment of asthma through a better understanding of the mechanisms associated with sub-optimal responses to current therapies. Support: GM121648, DoD W81XWH-17-1-0413, ES071431, and ES027015.

**3145 Role of Carrageenan in the Development and Prevalence of Inflammatory Bowel Disease**


Carrageenan which competitively inhibits Deleted in Malignant Brain Tumors 1 (DMBT1) mediated protection from bacterial invasion, preventing DMBT1 from suppressing inflammation. Carrageenan also interacts with other inflammation regulatory genes and proteins, such as cyclooxygenase-1, transmembrane serine protease 11, tumor necrosis factors, and interleukin 1 Beta. Binding to any of these targets either inhibits anti-inflammatory responses or produces cytotoxic effects at an unknown threshold, which subsequently increases inflammation. Through carrageenan’s broad binding specificity with multiple genes and proteins involved in mucosal homeostasis and inflammatory response, several molecular initiating events are shown to lead directly to adverse outcomes. This study shows the harmful effects of carrageenan with IBD: the ability to exert direct cytotoxic effects on intestinal epithelial cells, stimulate pro-inflammatory cytokines, interfere with innate mucosal immune responses, disrupt the epithelial barrier, and increase bacterial invasion. Further research focusing on specific food additives, such as carrageenan, and the microbiome to fully understand the relationships of the mechanism and pathways of IBD could optimize individualized patient treatment and dietary strategies not only for IBD but for other inflammatory diseases.

**3146 Mechanisms Driving Ectopic TRPV1 1585I/V-Induced TRPA1 Expression by Human Lung Epithelial Cells**


Asthma is a chronic inflammatory disease of the airways, and it is both caused by and exacerbated by environmental pollutants. Transient receptor potential ankyrin-1 (TRPA1) and vanilloid-1 (TRPV1) are sensors of diverse environmental pollutants including coal fly ash and diesel exhaust particles. Both TRPA1 and TRPV1 have been shown to be expressed at higher levels in epithelial cells and neurons of people with asthma, which may underlie hypersensitivity to environmental stimuli. Our previous studies demonstrated that the reduced function TRPV1 1585I/V SNP genotype correlated with poorer asthma symptomatology, a dramatic increase in IL-6 and IL-8, which are key pro-inflammatory cytokines in severe asthma exacerbations, whereas treatment of normal human lung epithelial cells with the I585I/V genotype following treatment with diesel exhaust particles, which activate TRPA1. A pharmacological approach has been developed to reproduce the effect of the loss-of-function TRPV1 1585I/V genotype on TRPA1 expression by HBECS and to probe mechanisms driving TRPA1 expression. Treating transfection of human bronchial epithelial cells of the I585I/V genotype with nonivamide reduced TRPA1 expression, while the TRPV1 antagonist LJO-328 increased TRPA1 expression at both the mRNA and protein/functional levels. Using RNA sequencing and qPCR, it was found that TRPV1 1585I/V-expressing HBECS also exhibited lower levels of expression of the nuclear factor kappa B (NF-κB) induced regulatory factor NACHT, LRR and PYD domains-containing protein 2 (NLRP2), which inhibits NF-κB. Direct inhibition of NF-κB with BMS-345541 decreased TRPA1 and NLRP2 expression suggesting that the basal activity of NF-κB is reduced when TRPV1 is less active. Additional results implicated MAPK and PKC in regulating TRPV1 function and TRPA1 expression. Specifically, direct inhibition of MAPK with PD98059 and PKC with Go6983 reduced basal TRPA1 expression in HBECS, while the PKC activator PMA reduced TRPA1, in part by promoting TRPV1 expression. Finally, NLRP2 targeted siRNA transfection into human bronchial epithelial cells will be used to determine if TRPA1 expression changes. Elucidating a mechanism for ectopic expression of TRPA1 by HBECS should advance our understanding of a phenomenon that may be a key risk factor for asthma pathogenesis. Support: ES017431, ES027015, and GM121648.

**3146a A Comparative Analysis of Salivary and Nasal Inflammation Biomarkers in Users of e-Cigarettes, Hookah, and Cigarettes**

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The effects of cigarette smoking on human health have been extensively investigated, but the impacts of other tobacco derived products, hookah and e-cigarettes, are less known. Saliva collection is the prevailing non-invasive method to detect a range of biomarkers in clinical and research settings. Recent advances in biological sample collection and subsequent analysis techniques have led to a non-invasive sampling method of the epithelial lining fluid (ELF) from the nasal passage. The ELF method collects a consistent volume, allowing quantification of low-abundance soluble biomarkers such as IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-18, INF-y, and TNF-a. These in-

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flamatory biomarkers can indicate the presence of chronic inflammation caused by cigarette, e-cigarette, and hookah use. Several studies have quantified inflammation marker levels in individuals utilizing either saliva or ELF, but no data have been published comparing saliva and ELF samples from the same individual for smokers or vapers. In this study, baseline samples of saliva and ELF were collected from 14 cigarette smokers, 20 e-cigarette vapers, 16 hookah smokers, and 31 non-smokers. Ten inflammation-related cytokines were quantified using the Meso Scale system. Seven of the biomarkers from the ELF of vapers showed significant differences from smokers and non-smokers, while no significant differences were seen in the salivary biomarkers.

3147 Quantitative Analysis of Quercetin in Hyperacanthus amoenus and Carissa bispinosa Foliages
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Sponsor: A. Adeogun
Pro-oxidant molecules such as reactive oxygen species are a major threat to the livestock across the globe. Their presence may arise in response to inevita-
able adverse environmental conditions such as poor nutrition, environmental pollution and UV radiation. These molecules trigger oxidative stresses result-
ing in high morbidity and mortality rates. Flavonoids are the largest group of phytonutrients, which play a significant role as strong antioxidants to prevent various diseases due to oxidative stresses. Hence, the objective of this study was to evaluate the commercial extract (P) of Hyperacanthus amoenus (P-A) and Carissa bispinosa (CF) foliages. The extracts were subjected to quantitative analysis of quercetin levels as putative anti-nutritive and toxic factors using BioTek microplate reader. The presence of quercetin was confirmed on HPLC-WVD system. The data was analysed using Mann-Whitney U statistical tool at a threshold level of 0.05. The quantification method was validated for linearity, accuracy, precision (repeatability, intra- and inter-day (3 days) vari-
ability), limits of detection (LOD) and quantification (LOQ). Accuracy and preci-
dasion were determined in terms of percentage and relative standard deviation (%RSD). The results showed LOD of 0.0071, 0.0185 and 0.0145 mg/ml in F, CB and HA, respectively. The inter-day recoveries for HA ranged between 100-103.2%, 71.9-84.9% and 82-106.8% at fortification levels of 0.25, 0.5 and 0.7 mg, respectively. The recoveries for CB ranged between 82-91.101%, 81.1-82.6% and 100-102.3% at 0.25, 0.5 and 0.75 mg fortification levels, respectively. While the LOQ for F varied between 88-90.88%, 90-119% and 97-112.2% at 0.25, 0.35 and 0.5 mg fortification levels respectively. The inter-day precision of F, CB and HA samples were within acceptable range according to CODEX Guidelines (%RSD<0.2). The quercetin contents were 0.073±0.042 (0.015%), 5.92±0.72 (0.59%) and 5.72±0.410 (0.57%) mg/g DW in F, CB and HA, respectively. These results were significantly different (p<0.05) and also confirmed on HPLC-WVD where the intra-day precision were found to be within the acceptable range (%RSD<15%). Therefore, the methods evalu-
ated indicate that relatively low quercetin contents in selected plants may not have serious adverse health and growth performance effects on livestock.

3148 The Testing of Lovage Extract (Levisticum officinale) to Meet the Requirements of Chemical Regulations
Ingredients for food use are exempt from general Registration, Evaluation, Authorization and Down Stream User (DSU) chemical safety assessment obligations, under chemical regulations e.g., Registration, Evaluation, Authorization and Restriction of Chemicals (REACH), EU or Registration, Evaluation, Authorization and Restriction of Chemicals in Turkey (KDKDIK). However, the use of such ingredients for non-food applications results in re-
porting obligations, the extent to which is directly related to the quantity of the used ingredients. These ingredients can be further assayed for activity against six clinical isolates of *S. aureus* which were resistant to at least two antibiotics. The number of bioactive compounds present in the extracts was evaluated using bioautography. Cytotoxicity of the eight extracts was determined on Vero and bovine dermis cells and selectivity index values calculated. MIC values of the extracts against resistant isolates of *S. aureus* ranged from 0.02 to 0.63 mg/ml. *M. frangula* extracts had the best activity (0.02 to 0.04 mg/ml), followed by *K. pinnata* extracts (0.04 to 0.08 mg/ ml). All the extracts had at least two bioactive compounds in bioautography with MIC values. All the extracts had at least two bioactive compounds in bioautography with *K. pinnata* extracts having the highest number of 7. Cytotoxicity results showed that *K. pinnata* extracts, the least cytotoxic extracts with selectivity index (SI) ranging from 12.01 to 24.58. The SI of other extracts ranged from 0.4 to 3.76. These results demonstrate that *K. pinnata* extracts, have non-cytotoxic and potentially interesting active compounds against mastitis and skin ulcer. Further work is ongoing to investigate the bioactive principles of these active extracts in therapeutic formulations.

3150 Virgin Coconut Oil Polyphenols Alter Epithelial-Mesenchymal Markers and the Migratory Potential of Melanocytes
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The process of epithelial cell differentiation to a mesenchymal phenotype, known as Epithelial-Mesenchymal Transition (EMT), is characterized by changes in the cell’s morphology, adhering capacity, and expression of spe-
cific surface protein markers. Virgin coconut oil (VCO) extracted from the co-
cconut (Cocos nucifera L.) is an emerging functional food with reported pharmacological properties. A natural combination of phenolic acids such as syringic acid, p-coumaric acid, ferulic acid, and kaempferol as revealed by LCMS-Q-TOF has been reported. Herein VCO (200µg/mL), its polyphenolic component (VCO 80 µg/mL), and VCO-less oil fractions (VCO 120 µg/mL) were individually exposed to metastatic B16F10 melanocytes in culture for 24 hours. The migratory potential of the control and exposed cells was assessed by wound healing and trans-well migration assays. It was observed that exposure of melanocytes to VCO, but not VCO or VCOF, reduced the migratory potential of the cells as against untreated controls. Further, when the mechanistic basis for the VCO induced inhibition of cell migration was sought, it was observed that the expression of typical surface protein markers N-cadherins was reduced while marginally increased E-cadherin in VCO exposed B16F10 melanocytes. On the other hand, matrix metalloproteinase-2 (MMP-2) did not change. Neither VCO nor VCOF addition altered the expression of these genes. The results show that VCO is effective in reducing the migratory potential of met-

3149 Activity and Cytotoxicity of Selected Medicinal and Alien Plants of South Africa against Wild-Type and Drug-Resistant Clinical Isolates of Major Causative Organism of Mastitis and Skin Ulcer
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Mastitis is an inflammation of the mammary gland and tissues of the udder or breast usually associated with skin ulcer and caused mainly by microbes. It is challenging to the health of humans and cattle due to antibiotic resist-
tance of *Staphylococcus aureus*, the major causative organism. In the dairy sector, mastitis causes huge financial losses due to medication difficulties. We investigated the antimicrobial activity of leaf extracts of 16 plants selected on the basis of preliminary activity of related species in the same family, or relevant traditional uses of the species. A broth microdilution assay using the acetone and ethanol extracts of these plants was used to determine activity against an ATCC strain of *S. aureus*. Among these 16 plants, extracts of four plants, namely *Mycteyes undata*, *Maurecinea frangula*, *Kalanchoe pinnata* and *Bryophyllum pinnatum*, had significant activity with minimum inhibitory concen-
tration (MIC) values ranging from 0.02 mg/ml to 0.32 mg/ml. These were further assayed for activity against six clinical isolates of *S. aureus* which were resistant to at least two antibiotics. The number of bioactive compounds present in the extracts was evaluated using bioautography. Cytotoxicity of the eight extracts was determined on Vero and bovine dermis cells and selectivity index values calculated. MIC values of the extracts against resistant isolates of *S. aureus* ranged from 0.02 to 0.63 mg/ml. *M. frangula* extracts had the best activity (0.02 to 0.04 mg/ml), followed by *K. pinnata* extracts (0.04 to 0.08 mg/ ml). All the extracts had at least two bioactive compounds in bioautography with *K. pinnata* acetone extract having the highest number of 7. Cytotoxicity results showed that *K. pinnata* extracts, the least cytotoxic extracts with selectivity index (SI) ranging from 12.01 to 24.58. The SI of other extracts ranged from 0.4 to 3.76. These results demonstrate that *K. pinnata* extracts, have non-cytotoxic and potentially interesting active compounds against mastitis and skin ulcer. Further work is ongoing to investigate the bioactive principles of these active extracts in therapeutic formulations.
3151 In Vitro Toxicity and Metabolism of a Potentially Sustainable, Fungal Colourant—Dermorubin

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The current industrially used colourants pose a significant occupational health risk and cause environmental complications upon their release to the environment within industrial effluents. In the future, colourants from biological matter may serve as a sustainable choice. However, their toxicity is scarcely studied. Dermorubin is a red anthraquinone colourant obtained from a fungal species Cortinarius sanguineus. We studied the toxicity of dermorubin in two human cell lines: human breast cancer line MCF-7 and a hepatic cell line HepG2. We evaluated the effect of dermorubin on cell viability with MTT and propidium iodide (PI)-diginiton assays, cytotoxicity with lactate dehydrogenase test, production of reactive oxygen species with dihydrodichlorofluorescein diacetate assay, and superoxide formation both in cytosol with dihydroethidium assay and mitochondria with mitochondrial superoxide assay. We used six dermorubin concentrations from 0.03 to 10 µg/ml, and respective positive and negative controls. The experiments were repeated three times and each concentration had four replicates. Cells were seeded onto 48-well plates 24 h prior to exposure and then exposed to dermorubin for another 24 h. Dermorubin did not show any toxicity in concentrations up to 10 µg/ml in MCF-7 cells. MTT and PI-diginiton assays were conducted in HepG2 cell line and no toxic effects were observed. In addition, dermorubin was not mutagenic in a miniaturized Ames test. Further, we studied the metabolism of dermorubin in human liver microsomes and cytosol which contain the metabolic enzymes UDP-glucuronosyltransferase, sulfotransferase, cytochrome P450 enzymes, and catechol-O-methyltransferase which catalyse glucuronidation, sulfonation, oxidation, and methylation reactions, respectively. Our results show that dermorubin is not a substrate for any of these enzymes, but further studies in a single-enzyme assay are needed. In conclusion, our results show that dermorubin could potentially reduce the current flux of chemically very stable colourants into the environment. This study is a part of the BioColour project that aims to identify biodegradable and sustainable colourants that are safe for humans as well.

3154 The Role of Mitochondrial Complex II Activity on RAW 264.7 and IC-21 Macrophages Response to Crystalline Silica


Macrophages (Mφs) differ significantly in their response to crystalline silica. Previous studies reported that RAW 264.7 and IC-21 Mφs cell lines, which reproduce the effects of silica on primary mouse Mφs derived from BALB/c and C57BL/6J mice respectively, differ significantly in their viability and cytokine response to silica. While RAW 264.7 Mφs release TNFα and exhibit low cell cytotoxicity in response to silica, IC-21 Mφs do not release TNFα and experience universal cell death, even at low silica dose. Therefore, to better understand the role of mitochondrial complex II (CII) on Mφ survival, the effect of silica on CII enzymatic activity on RAW 264.7 and IC-21 Mφs were compared. Unlike Mφs RAW 264.7 and IC21 were exposed to silica (50 µg/cm²) with or without priming with LPS (1 ng/ml); metabolic parameters were assessed at increasing time point up to 24 hours after silica exposure. Compared to baseline, LPS did not alter RAW Mφ viability, and was as effective as silica in promoting lactate release, while addition of silica to LPS-primed cells induced a greater increase in lactate release. Moreover, silica or LPS-treated Mφs experienced 30% necrosis, while primed Mφs exposed to CS up to 70% necrosis, assessed by FITC Annexin and PI staining. In sharp contrast, silica, not LPS, induced significant cell cytotoxicity and LDH release in IC-21 Mφs; silica addition to CII enzymatic activity on RAW 264.7 and IC-21 Mφs revealed a synergistic effect that reduced the cell survival to 5% at 24h post-exposure. However, LPS stimulation of IC-21 Mφs enhanced the lactate release more than silica. Using a high-resolution respirometer, the effects of silica on mitochondrial respiration, and activity, and integrity of the electron transport chain (ETC) were assessed in both cell lines. Interestingly, succinate stimulation of silica-treated RAW 264.7 Mφs revealed an enhancement in O₂ consumption and CII enzymatic activity (while decreasing complex I, Cl activity), greater than LPS-primed. Conversely, succinate stimulation of CII did not affect the O₂ consumption of LPS-primed IC-21 Mφs, but silica alone inhibited CII activity in IC-21 as early as 2 hours post-exposure, even after LPS priming (while silica did not affect Cl activity). In conclusion, RAW 264.7 and IC-21 Mφs cell lines reported significant differences in immuno-metabolism.

3153 Nephrohepatoprotective Ability of Fractions of Ageratum conyzoides in Carbon Tetrachloride-Induced Rats

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Medicinal plants are unique sources for both anti-hepatotoxic and anti-nephro-toxic agents. They are basically needed to reduce the susceptibility of these vital organs (liver and kidney) to severe damages and also onset of cancer. For this, medicinal utilizes Ageratum conyzoides in the treatment of wound, burns and increasing lactation of nursing mothers and has also been assumed to have organo-protective effects. Several natural bio-agents are increasingly utilized to combat pathological damages induced by chemicals because of their effective roles in ameliorating oxidative stress and thereby sustaining the organ(s). The present study evaluated the protective effect of A. conyzoides against carbon tetrachloride-induced toxicity in the liver and kidney of rats by utilizing thirty rats. The effect of various fractions (Chloriform, Ethyl-acetate and Methanol) of crude methanol extract of A. conyzoides (200 mg/kg each) and silymarin 100 mg/kg against carbon tetrachloride - CCl₄ (2 ml/kg)-challenged rats were investigated by estimating the levels of antioxidant enzymes (GPx, GSH, SOD, CAT) liver enzymes found in the serum (γ-GT, ALP, ALT and AST). Other biochemical indices assessed were MDA (an oxidative stress biomarker), TB, CRT, TP and TC alongside histopathological examination on the liver and kidney of the rats. In the group that were pre-treated with various fractions of A. conyzoides the drastic reduction in the levels of GPx, SOD and CAT induced by CCl₄ was prevented. The significant (P<0.05) increase in the level of serum enzymes caused by CCl₄ were suppressed in the presence of various fractions of A. conyzoides. Although CCl₄ was able to significantly (P<0.05) increase the activities and levels of MDA, GST, TB, TG, TC but lowered the values of the A. conyzoides desirable effect than silymarin in reversing these adverse effect. These results submit that A. conyzoides stimulates protective ability against CCl₄ by its appropriate antioxidant activities.
in response to silica and LPS, and given the role of CII as a component of the Krebs cycle and ETC, its activity becomes a key regulator of M0s' immune response and survival.

3155 Investigating the Effect of Clerodendrum volubile Extract on Doxorubicin-Induced Toxicities: In Vivo and In Silico Studies

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Doxorubicin (DOX) is a commonly used chemotherapeutic drug. However, its non-target organ toxicities pose a serious problem. This study is to assess the protective role of Clerodendrum volubile leaf extract (CVE) against DOX-induced toxicities in rats. In addition, the inhibitory activities of three phytochemical compounds (Rutin, Gallic acid and Rosmarinic acid) from CVE against Carbonyl reductase 1 (CBR1) were examined. Rats were randomly divided into five groups: (a) Control group rats were given 0.9% NaCl as vehicle, (b) DOX group: A single dose of DOX (25 mg/kg, i.p.) was administered and rats were sacrificed 4 days after DOX injection, while groups (c-e) CVE-treated DOX rat groups were given 125, 250 and 500 mg/kg body weight of extracts orally for 12 consecutive days; 8 days before, and 4 days after the DOX administration.

Computational techniques were used to determine the inhibitory activities of the compounds against CBR1. DOX intoxication caused a significant increase in the ratio of P < 0.05) decreased in DOX-intoxicated rats with concomitant elevation of malondialdehyde levels. Pretreatment with CVE reversed the above trends. From the structural analysis, Rutin and RSA exhibited the highest binding free energies against CBR1, and also exhibited structural stability when bound with CBR1. Our study indicates the protective effect of CVE when used in combination with doxorubicin thus improving its chemotherapeutic application via inhibition of CBR-mediated metabolism.

3157 The Synergetic Oxidative Stress in A549 Cells Induced by Arsenic Solution As (III) and Cigarette Smoke Condensates

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Arsenic is classified by the International Agency for Research on Cancer (IARC) and the US Environmental Protection Agency (EPA) as a known human carcinogen. Previous studies have demonstrated that active smoking and exposure to arsenic act synergistically to increase the risk of lung cancer. However, the mechanism leading to synergism of arsenic and smoking is not clear. Oxidative stress may play an important role in disease pathogenesis. In this study we investigated oxidative stress in A549 cells induced by arsenic acid solution As (III) and cigarette smoke condensates (CSC) and study their interaction. A549 cells were divided into 4 groups: arsenic acid solution As (III) treated group (dose of As(III) and CSC: 0.88 μg/mL and 0 μg/mL respectively), cigarette smoke condensates treated group (dose of As (III) and CSC: 0.88 μg/mL and 75 μg/mL respectively), arsenic acid solution As (III) and cigarette smoke condensates treated group (dose of (As (III) and CSC: 0.88 μg/mL and 75 μg/mL respectively), and control group (dose of As (III) and CSC: 0 μg/mL and 0 μg/mL respectively). The viability of A549 cells was tested by the CCK-8 assay, and two indicators of oxidative stress, reactive oxygen species (ROS) and extracellular superoxide dismutase (EC-SOD) were determined respectively. 2×2 factorial design was used to evaluate the interaction. Results showed that relative fluorescence intensity of ROS and concentrations of EC-SOD were (1.01±0.03), (0.30±0.01) ng/mL in arsenic acid solution As (III) treated group; (3.88±1.02), (0.33±0.04) ng/mL in cigarette smoke condensates treated group; (5.45±1.31), (0.77±0.11) ng/mL in arsenic acid solution As (III) and cigarette smoke condensates treated group, respectively, those were significantly higher than those in control group (1.00±0.03), (0.29±0.03) ng/mL. The results of the factorial design showed arsenic acid solution As (III) and cigarette smoke condensates had interaction on ROS production and EC-SOD levels in A549 cells, the interaction was synergism. These results could be important to further reveal the mechanisms of the synergy of arsenic and smoking.

3158 Real-Time Monitoring of Redox Events in Human Airway Epithelial Cells Exposed to an Environmental Peroxide

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Exposure to air pollutants such as ozone, particulate matter, and secondary organic aerosols induces intracellular oxidative stress. Reactive oxygen species play an essential role in maintaining intracellular redox balance by regulating critical signaling pathways. Intracellular redox homeostasis is maintained by a high ratio of reduced to oxidized glutathione (GSH and GSSG, respectively), which is tightly regulated with reducing equivalents such as NADPH. In this study, we investigated the effect of ISOPOOH, a known constituent of secondary organic aerosols, and cellular response to environmental peroxides such as ISOPOOH, a known constituent of secondary organic aerosols induces intracellular oxidative stress. Reactive oxygen species play an essential role in maintaining intracellular redox balance by regulating critical signaling pathways. Intracellular redox homeostasis is maintained by a high ratio of reduced to oxidized glutathione (GSH and GSSG, respectively), which is tightly regulated with reducing equivalents such as NADPH. In this study, we investigated the effect of ISOPOOH, a known constituent of secondary organic aerosols, and cellular response to environmental peroxides such as ISOPOOH. Exposure to ISOPOOH induced oxidation of intracellular glutathione that was markedly potentiated by glucose deprivation. Spontaneous recovery of E GSH was observed initially but not upon exposure to higher ISOPOOH concentrations, suggesting irreversible changes with sustained oxidative stress. Strikingly, addition of 1 mM glucose following ISOPOOH challenge induced rapid and complete restoration of E GSH, which was accompanied by increased NADPH levels, as reported by iNAP1. Furthermore, ISOPOOH-induced changes in E GSH and NADPH did not lead to increased intracellular H2O2, as measured by HyPer, suggesting that induction of E GSH by ISOPOOH is independent of intracellular H2O2. These findings highlight the early mechanisms involved in the cellular response to environmental peroxides such as ISOPOOH and provide an unprecedented live view of the dynamic regulation of redox homeostasis in human lung cells. Does not reflect US EPA policy.
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3159  Elevation of Cellular Glutathione Inhibits Neuronal Hyperexcitability via Redox Regulation of mTOR
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Exposure to neurotoxicants like organophosphate pesticides, nerve gas agents and heavy metals can cause recurrent, uncontrolled seizure activity (neuronal hyperexcitability). Oxidative stress and a perturbed glutathione (GSH) redox status are heavily implicated in the pathogenesis of toxicant-mediated seizure activity. However, whether and how GSH redox modulation regulates neuronal hyperexcitability is unclear. We asked if modulation of cellular neuronal redox status with a thiol-containing compound would attenuate neuronal hyperexcitability in vitro and in vivo. We have previously shown that 2,3-dimercapto-1-propanol (DMP), a thiol-containing drug significantly increases intracellular GSH levels in rat primary neuronal-glial cultures by a novel mechanism: post-translational activation of the rate-limiting enzyme in GSH biosynthesis. In the current study, we determined if increased GSH levels would attenuate neuronal hyperexcitability in vitro and in vivo. Pre-treatment of neuronal-glial cultures with DMP increased GSH levels (measured by high performance liquid chromatography, HPLC) and significantly decreased 4-aminoypyridine ([AP], toxicant that blocks voltage-gated potassium channels) induced neuronal hyperexcitability (measured by microelectrode array). Next, we tested transgenic Dravet Syndrome (DS) zebrafish larvae that exhibit convulsive ‘seizure-like’ swim behavior with DMP. DMP elevated GSH levels and decreased ‘seizure-like’ swim behavior (measured by Noldus locomotion assay) in DS larvae. Next, we asked if the redox-sensitive mammalian target of rapamycin (mTOR) pathway is the mechanistic link between GSH redox status and hyperexcitability. Indeed, cultures treated with buthionine sulfoximine (BSO, to deplete GSH) or with 4AP showed aberrant activation of mTORC1 (assessed by pS6/S6 levels by immunoblotting). Surprisingly, cultures pre-treated with DMP and then with BSO or 4AP were protected against mTORC1 hyperactivation (measured by redox western blotting) in cultures treated with BSO revealed >25% oxidation of hamartin (TSC1) and tuberin (TSC2), critical negative regulators of mTORC1 kinase activity. Taken together, the data suggest that DMP improves GSH redox status and controls neuronal hyperexcitability in vitro and in vivo and one possible mechanistic pathway involved is mTOR. Support: NIHNS R01NS086423 (M.P.).

3160  An Effect-Based Comparison of Conventional Drinking Water Production and Pilot-Scale Ozonation and Granular Activated Carbon Filter Treatment: Special Focus on Oxidative Stress and Mutagenicity
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Conventional drinking water treatment plants (DWTPs) are designed to provide clean drinking water primarily safe from harmful pathogens; however, such methods may not completely eliminate micropollutants in the treated water. As such, there is a need for vital research into the effectiveness of existing drinking water treatment methods in the development of newer technologies. In the current study, a panel of cell-based bioassays were used to assess a pilot-scale treatment system e.g., ozonation and granular activated carbon (GAC) filtration vs. a conventional full-scale DWTP that employed coagulation, sedimentation, sand filtration, biological activated carbon filtration, UV disinfection and dosing with bromochloramine and chlorine. Three sampling events were completed over the course of 10 months. The endpoints of interest were oxidative stress (Nrf2 activity), mutagenicity (micronuclei formations), aryl hydrocarbon receptor (AhR) activation, and androgen receptor (AR) activation and inhibition. Compared to the full-scale treated samples, lower Nrf2 and AhR bioactivities and micronuclei formations were observed in the pilot-scale samples across all sampling events. These findings highlight the effectiveness of the combination of ozonation and GAC filtration in reducing bioactive compounds in drinking water treatment. In comparison, higher bioactivities were detected in the full-scale samples and variability was observed between the sampling events. This suggests that conventional drinking water treatments may not be entirely effective in removing compounds that induce bioactivities. For the AR assay, none of the samples collected from both treatment systems showed any androgen agonist or antagonist activities. The conclusions made from the bioassay results, regarding both the pilot-scale and full-scale drinking water treatment methods, provide important insights into the optimization of existing drinking water treatment designs and utilization of alternative treatment technologies.

3161  Ethanol Perturbs Collagen Synthesis and Mineralization in Bone, Associated with Impaired Periocular-Canalicular Remodeling
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Chronic alcohol consumption during early adulthood impedes the achievement of peak bone mass, which greatly predisposes to osteoporosis and osteoporotic fractures in both genders. Further, ethanol (EtOH) compromises bone’s mechanical properties (elasticity, stiffness, load-carrying capacity, and toughness), which influence bone quality. The molecular mechanism is not currently understood. In bone, osteocytes are the main regulators of quality. EtOH is known to impact osteocytes: for instance, it induces osteocyte apoptosis. We are interested in investigating how EtOH affects osteocytes to render bone fragile. We hypothesize that EtOH interferes with osteocyte-mediated bone remodeling, a process known as periocular-canalicular remodeling (PLR), which is essential for collagen organization and bone matrix mineralization. We performed a 4-day binge ethanol study where 12-week-old mice were gavaged with saline vehicle or 31.5% (v/v) EtOH in water at a dose of 3 g/kg, 3 g/kg, 4 g/kg and 4.5 g/kg (peak BAC approx. 0.2%) on four consecutive days and sacrificed 6 h following the final dose. We also performed in vitro studies with a novel osteogenic cell line (OmgFP66) which we exposed to 50 mM EtOH either acutely (24 h prior to harvest) or chronically (throughout the entire differentiation). Transcriptomes of male mice femoral shaft analyzed by RNA-Seq were compared between treated and control mice and revealed significantly increased transcription of the key PLR mediators MMP13 and Ctsk by 1.8- and 1.3-fold, respectively, as well as decreased expression of several collagens, including Col1a1 and Col1a2, in the EtOH-treated groups. In vitro studies with 24-hour ethanol exposure of fully differentiated OmgFP66 cells (>28 days of differentiation) replicated these findings, revealing similar fold increases in MMP13 and Ctsk in the alcohol-treated group. Furthermore, osteogenic differentiation in vitro in the presence of 50 mM EtOH for 21 days disrupted the mineral bone spicules, as observed by confocal microscopy following Alizarin Red staining. We conclude that ethanol perturbs collagen expression and bone mineralization, but whether it does so through impairment of PLR remains to be proven. Funded in part by R37 AA018282 (M.R.).

3162  Reduced Osteoblast Function Is a Feature of Both Chronic and Binge Ethanol Exposure in Mice
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Alcohol abuse is a risk factor for development of osteoporosis. Excessive ethanol (EtOH) use decreases bone density and weakens bones’ mechanical properties. Ethanol can inhibit bone formation and stimulate bone resorption in association with development of oxidative stress. Chronic ethanol consumption for 3 months leads to reduced expression of genes for major bone matrix proteins in the mouse femur shaft independently of NADPH oxidase 4 (Nox4) (IPEF 373:337-346, 2020). To investigate short-term effects of EtOH, we exposed wild type and catalase knockout (KO) mice to a 4-day ethanol binge exposure (3, 3, 4 and 4.5 g EtOH/kg). While catalase KO increased the level of liver triglycerides in EtOH-exposed animals (P<0.05), it had no effect on the EtOH-mediated decrease in bone formation as determined by serum osteocalcin. To identify genes regulated by both chronic and binge ethanol, we compared the femoral shaft transcriptome analyzed by RNA-Seq from the chronic and acute ethanol exposure studies for an analysis of the main effects of ethanol independently of the Nox4 or Catalase genotypes. 325 genes were significantly downregulated by both chronic and binge EtOH, of which at least 60 genes are involved in collagen synthesis and osteoblast function. They include collagen genes, including Col1a1 and Col1a2, that are the two highest expressed genes in both studies, lysyl oxidase genes Lox, Loxl2, Loxl4 for generating lysine-derived collagen crosslinks, prolly 4-hydroxylase genes P4ha2 and P4hb for generating the hydroxyproline in the collagen polypeptides, the ER-specific collagen chaperone Serpinh1 as well as genes such as Lbp and Sparc encoding other extracellular matrix proteins. We finally investigated gene expression from lumbar vertebrae of control mice of both sexes exposed to chronic ethanol for 2 months by qRT-PCR. There was significantly reduced expression of Blapg, Col1a1, Col2a1, Col5a2, Col1a1, Chtrc1, Lox, Mmp16, Smd5 and Sparc. We conclude that EtOH exposure in mice reduces osteoblast function at the mRNA level in several bone types. Funded by LSHSC, PREP R25GM12189, and NIH-R37AA018282 M.RJ.
**3163** Dioxin Disrupts Thyroid and Glucocorticoid Hormone Induction of klf9, a Master Regulator of Frog Metamorphosis

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Frog metamorphosis, the transition of an aquatic tadpole to an air-breathing frog, is driven by prolactin, glucocorticoids (GC) and thyroid hormones (TH). It is susceptible to disruption by 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), an aryl hydrocarbon receptor (AhR) agonist, in a tissue-specific manner. A master regulator of metamorphosis, the transcription factor Krüppel-Like Factor 9 (klf9) is synergistically induced by GC and TH. This process is mediated by an enhancer cluster in the klf9 upstream region, the klf9 synergy module (KSM) which is highly conserved throughout the tetrapod lineage. klf9 is also a target of the AhR. We measured klf9 mRNA expression following combined exposures to triiodothyronine (T3), corticosterone (CORT), and TCDD in the Xenopus laevis cell line XLK-WG. klf9 was induced 6-fold by 50 nM T3, 3-fold by 100 nM CORT, 2-fold by 175 nM TCDD. Combined treatment with CORT plus TCDD or T3 plus TCDD respectively elicited a greater-than-additive 9- and 10-fold induction, while co-treatment with all 3 agents induced klf9 mRNA 16-fold. We next performed luciferase reporter gene assays to examine regulatory sequences from the Xenopus tropicalis klf9 upstream region, testing the hypothesis that AhR regulates klf9 expression by interacting with the KSM. A high fraction of predicted sequences containing the KSM were associated with a strong T3 response and a larger combined T3/CORT response, but induction by TCDD was instead mediated by a region ~1 kb farther upstream. Unexpectedly, this region also supported a robust CORT response. To better understand the mechanisms of these interactions, we performed transactivation assays with mutant klf9 regulatory sequences, identifying 5 AhR response elements of varying strength. The non-KSM region that supported a GC response did not contain a readily-identifiable glucocorticoid responsive element (GRE), suggesting that the response may be mediated by protein-protein interactions. Our results indicate that klf9 is regulated by multiple overlapping mechanisms, and that its endocrine control is vulnerable to disruption by TCDD. NIH R15 ES011130.

**3164** In Utero and Lactational Exposure of Mice to 2,3,7,8-Tetrachlorodibenzo-p-dioxin Results in Toxic Skin Effects


Early-life exposures to persistent organic pollutants (POPs) impact developmental and alter neurological, cardiovascular and reproductive functions in later-life. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a prototype in a family of POPs, the dioxin-like halogenated compounds (DLCs), that mediates its toxic effects by activating the aryl hydrocarbon receptor (AhR). Mice with constitutively active AhR in keratinocytes develop atopic dermatitis (AD)-like symptoms in their lifetime. Additionally, topical TCDD application results in murine sebophytosis, implicating sebaceous progenitor cells in the toxicity of TCDD. We previously reported that TCDD-exposed fetuses have accelerated epidermal barrier development in utero. In this study we investigated the effects of perinatal exposure to TCDD on the development of epidermal barrier and susceptibility to AD in C57BL/6J mice. Mice were exposed in utero to TCDD at a dose of 5 μg/kg bw on embryonic day 12 and the effects on barrier formation and function were studied from postnatal day 1 (P1) through adult life. Pups exposed to TCDD were born with diffuse epidermal hyperplasia; however, this effect did not persist in adult life. TCDD-exposed animals followed from birth to P135 did not develop any AD-related pathology such as skin lesions, increased serum IgE and Th2 immune response. Further, control and exposed adult animals developed the same level of skin inflammation in response to MC903, a vitamin D analogue, indicating that TCDD did not cause long-term immune derangement.

Sebaceous gland hyperplasia, reminiscent of chloracne, was found in TCDD-exposed P21 mice. CYP1A1 was detected only there, while CYP1B1 was and immunoglobulin-like domains protein 1 (LRIG1)-expressing progenitor cells at the infundibulum. CYP1A1 was detected only there, while CYP1B1 was expressed throughout the epidermis, sebaceous glands, and isthmus, indicating multiple cell-type targets of TCDD. In conclusion, perinatal exposure to TCDD results in a chloracne-like phenotype in the skin, without evidence of atopy. The identity of specific skin cells affected by TCDD will aid in the continued research into the mechanisms of chloracne which include sebaceous gland dysmorphogenesis and epidermal hyperplasia.

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**3165** Collective Characterization of Pregnancy X Receptor Activation by Gut Microbiome-Derived Compounds

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The mammalian pregnant X receptor (PRX) regulates the transcription of several xenobiotic metabolizing enzymes, including the important 3A family of cytochrome P450s (CYP). Recent studies have shown that metabolites produced by the gut microbiome (e.g., indoles, secondary bile acids) induce PRX transcriptional activation. In the present work, we used a cumulative approach to identify the impact of gut microbiome-derived compounds on PRX expression and transcriptional activation. Liver, duodenum, jejunum and ileum were harvested from germ-free (GF) and conventional (CV) C57BL/6J mice, and the transcript levels of Prx and PRX regulatory targets were assessed by RT-qPCR. Tissue-specific differences in PRX expression were observed, with GF mice having significantly reduced Prx levels compared to CV in the liver (0.66x, p<0.05, t-test), but increased in the duodenum (9.1x, p<0.001) and ileum (3.7x, p<0.05). In the liver of GF mice, reduced Prx expression also corresponded to decreased expression of the PRX target genes Cyp3a11 (0.25x, p<0.0001), Gstm1 (0.38x, p<0.001), Gstm2 (0.33x, p<0.01), and Papp2 (0.43x, p<0.001). Similarly, upregulated Prx in the duodenum of GF mice was concurrent with significant increases in Cyp3a11 (14.0x, p<0.0001), Cyp2b10 (6.3x, p<0.001), Aldh1a1 (6.0x, p<0.01), Gstm1 (3.2x, p<0.001), and Gstm2 (3.7x, p<0.01). To determine whether microbe-produced compounds from human stool or mouse cecal contents could activate human PRX (hPRX) or mouse PRX (mPRX), respectively, PRX luciferase reporter cells were administered metabolites extracted with chloroform:water (1:1). Of 19 human fecal extracts, 4 significantly (p<0.05, ANOVA with Fisher’s LSD test) induced hPRX activation, with a range of 2.2-2.9x increases compared to control. 5 out of 20 mouse cecal content extracts activated mPRX, with significant fold-increases ranging between 1.5-3.8x. In summary, the gut microbiome is critical for the specific expression of PRX and PRX targets in the liver and small intestine and we observed individual variation in the PRX activation capabilities of compounds produced in the mammalian gut. Future work will be on identifying the individual PRX ligands in human stool, and mouse cecum and blood, using HPLC fractionation of extracts and untargeted LC-MS/MS. The unique composition and metabolic activity of the gut microbiome may be an important factor in the interindividual variability in PRX activity.

**3166** Flavonoids Are Nuclear Receptor 4A1 (NR4A1, Nur77) Ligands That Act as Inhibitors of Endometriosis

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Endometriosis is an inflammatory disease that primarily affects women during their reproductive years, and since current hormonal therapies are of concern, new hormone-independent treatment regimens are needed. Studies in this laboratory have identified and characterized the orphan nuclear receptor 4A1 (NR4A1, Nur77) as a novel drug target in hormone dependent (i.e. estrogens) cancer and other solid tumors including prostate, colon and endometrioma-sarcoma. Results of RNA interference (RNAi) studies demonstrate that NR4A1 is pro-oncogenic and regulates several genes/pathways that are important for cancer cell proliferation, survival and migration/invasion. The orphan nuclear receptor 4A1 (NR4A1, Nur77) is expressed in patient-derived (stromal and epithelial) endometriotic cells and we observed that knockdown of NR4A1 in patient-derived ectopic endometrioma-derived compound liable immortalized human endometrial stromal cells (LHESCs) and luciferase stable immortalized human endometrial epithelial cells (LIHEECs) decreased cell proliferation. Moreover, the treatment of these cells with the flavonoids such as Quercetin and Kaempferol inhibited cell growth and related genes. The compounds exhibit NR4A1 antagonist activities in both functional and transactivation assays whereas these effects were not observed in normal endometrial cells. We also observed that NR4A1 knockdown and treatment with NR4A1 inhibitors decreased fibrosis, oocyte maturation and related pro-fibrogenic genes in LHESCs and LIHEECs and similar results were observed in epithelial-derived Ishikawa cell lines. In this study we used both RNAi to knockdown NR4A1 and NR4A1 ligands (anagonists) to investigate their effects on endometriosis cell growth and survival and their impacts on mTOR signaling, a pathway that is targeted in treatment of endometriosis. This approach demonstrates the potential activation of Liver, duodenum, jejunum and ileum and also show that the Quercetin and Kaempferol act as antagonists and inhibit cell growth and mTOR signaling. Thus, NR4A1 antagonists represent a novel class of drugs as nonhormonal therapy for endometriosis.
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 AhR-ARNT target genes. Early studies have shown that AhR-deficient mice have altered lymphocyte numbers in the spleen, thus showing the importance of AhR for homeostasis of the immune system. In fact, AhR has been found to be functionally up-regulated during T cell activation and promotes the expression of the pro-inflammatory cytokine IL-22. The immune system requires a high degree of diversity to adapt and respond to changes in the environment. During T cell activation, about 10-15% of alternative exons undergo a >10% change in inclusion which is critical for eliciting an immune response. The main binding partner of AhR, ARNT, is alternatively spliced to produce two main isoforms, ARNT isoform 1 and 3, which differ in the inclusion of exon 5 found in ARNT isoform 1. Notably, naïve lymphocytes have equal amounts of ARNT isoform 1 and 3, whereas during T cell activation there is an increase in the amount of ARNT isoform 1. Through targeted suppression of individual ARNT isoforms it was found that the loss of ARNT isoform 3 during T cell activation promoted an increase in the expression of the T cell activation marker, IL-2. The NF-κB subunit, c-Rel, is an essential transcription factor required for the expression of IL-2 during T cell activation, and targeted suppression of both ARNT isoform 3 and c-Rel inhibits the increase in expression of IL-2. In naïve T cells, the chromatin of IL-2 is in a condensed state which becomes open during activation, allowing c-Rel to bind and promote IL-2 expression. However, in naïve T cells where ARNT isoform 3 is suppressed, ChiP analysis shows c-Rel is able to bind to the IL-2 promoter prior to activation, suggesting that the ARNT isoforms are involved in chromatin remodelling of the IL-2 promoter. This study has identified that a specific alternative splicing pattern of ARNT is critical for T cell activation and suggests that modulation in the ARNT isoform ratio may allow T cell adaptation to various environmental cues.

A differential roles of ARNT isoforms during T cell activation.

Differential Roles of ARNT Isoforms during T Cell Activation

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Virucidal Activity of Chlorine Dioxide Gas for Reduction of Coronavirus on Surfaces and PPE

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A coronavirus (SARS-CoV-2) has caused a global pandemic and associated morbidity and mortality resultant from COVID-19. As a result of efforts to control direct (person to person) and indirect (contaminated objects, surfaces, indoor air) transmission of the virus, various interventions have been evaluated. Studies were conducted to evaluate the efficacy of commercially available chlorine dioxide (CD) products to reduce viral loads on PPE (face masks) and surfaces using a novel dry gas release intervention. The efficacy of CD slow release 30-day sachets was tested on N95 face masks inoculated with human coronavirus OC43 in suspension based on adaptation of ASTM E1053. One sachet was placed with an inoculated mask in plastic resealable bags. Three trials were completed using the original sachet where a mask and sachet were placed into a plastic bag for 13 hours per sachet age of 1 day, 14 days, and 30 days. The amount of CD generated during a 13-hour treatment period was 0.30 mg. The plastic bags were quartz size (0.000946 m3). The nominal concentration of CD was estimated to be 317 mg/m3. All three tests demonstrated at least a 99.91% reduction of viral loading in the mask versus a non-treated control. Efficacy of CD dry gas fast releasing pods (Ultrashock) for fumigation was also tested in a 134 ft3 closed room based on adaptation of ASTM E1053. Two pods were placed in the space and CD surface virucidal efficacy was tested in three locations of the room after 1 hour and 2 hours of dwell time. The estimated nominal peak concentration was 15 ppmv in the room. The one-hour exposure saw a 99.999% OC43 reduction on surfaces and the two-hour exposure resulted in a >99.997% OC43 reduction on surfaces versus a non-treated control. These results indicate CD is highly effective against human coronavirus. CD was 99.91% effective for eliminating human coronavirus OC43 in both sachet and capsule fumigant form using both fast and slow release mechanisms. Rapid fumigant application is suitable for contaminated rooms, ambulances, emergency vehicles, and many types of PPE, most particularly porous PPE materials. The gaseous state of CD allows for rapid diffusion and transfer of the virucidal stable free radical to all surfaces of PPE and indoor areas that would favor virus survival. Additionally, this work suggests CD can be effective at levels with significant margins of safety (little to no exposure and rapid degradation of residuals) providing minimal public health risks associated with the use of CD.

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system has revolutionized biological research, allowing scientists to manipulate DNA with unprecedented ease and precision. This powerful genome-editing tool finds application in multiple fields of research including toxicological sciences, where the CRISPR/Cas9 technology can be harnessed to decipher the exact mechanism underlying toxicity. Yet, while widely applied to mammalian cell lines, limited knowledge is available on genetic manipulation in cell lines of fish. Thus, we developed a gene editing approach based on the CRISPR/Cas9 system in a rainbow trout (Oncorhynchus mykiss) cell line (RTgutGC). The cryp1a1 gene, encoding for the Cytochrome P450, family 1, subfamily A, polypeptide 1 was selected as first target. Ribonucleoprotein (RNP) complexes, consisting of Cas9 protein and a tracr/cRNA duplex, targeting the cryp1a1 gene, were directly delivered into RTgutGC cells via electroporation. T7 Endonuclease 1 (T7E1) assay performed on imprinted clinical outcome. Previous studies have examined immediately after cryp1a1 at the cryp1a1 locus. Sanger sequencing of the cryp1a1 gene followed by bioinformatic analysis further supported successful manipulation of the target site. A novel approach for clonal isolation based on low cell density seeding and the use of cloning rings was developed in RTgutGC cells. By doing so, two cryp1a1 mutant clones, bearing a 101 or 1 base pair insertion in the DNA region targeted by the RNP complex, were raised. This is the first report of a clonal CRISPR/Cas9 gene-edited fish cell line in rainbow trout.

Novel Proteomic Biomarkers for the Prediction of Renal Recovery from Dialysis-Dependent AKI Patients

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Acute kidney injury requiring dialysis (AKI-D) is associated with prolonged length of stay, mortality, and progressive chronic kidney disease (CKD) among survivors. Functional renal recovery leading to discontinuation of dialysis is an important clinical outcome. Previous studies have examined few urine or serum biomarkers to predict renal recovery from AKI; however, the performance of biomarkers of renal recovery has not been established. To identify new serum biomarkers that predict renal recovery from AKI-D, study day 8 samples from 72 patients enrolled in the Veteran’s Affairs/National Institutes of Health Acute Renal Failure Trial Network study were analyzed by the slow off-rate modified aptamers scan (SOMAscan) proteomic platform to profile 1305 proteins in each sample. Of these patients, 38 recovered kidney function and dialysis was discontinued, while the other 34 patients remained on dialysis by day 28. Changes in the serum levels of 119 proteins, 53 of which increased and 66 of which decreased, were detected when comparing samples of patients who were taken off dialysis versus patients who remained on dialysis by day 28. Serum levels of mortality-associated proteins, such as FGF23 and IL-6, were reduced in those that recovered, which was corroborated by Olink analysis. Some of these protein level changes included Wnt-7a, c-Myc, kinase BTK, epidermal growth factor (EGF), CXCL2/CXCL3, CXCL11, platelet-derived growth factor C, and survivin, when stratified by tertiles, and were associated with renal recovery by logistic regression analysis. The results suggest concerted changes between levels of immune response cytokines and recovery-related proteins that might play a role in regulating glomerular integrity, angiogenesis and tubular repair, illustrating potential mechanisms of kidney recovery. Thus, this study identifies potential novel predictive biomarkers of renal recovery from AKI-D patients.
3171 Multi-Frequency Impedance Cell Monitoring for Label-Free and Time-Resolved Cell Toxicity Analysis

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Reading the impedance of planar gold-film electrodes that are used as growth substrate for adherent cells reveals changes in cell morphology. Thus, real-time impedance data provide insights in various cell phenotypes such as cytotoxicity even over prolonged periods of time. A crucial advantage over standard microscopy-based continuous cell monitoring is the continuous information content is obtained by using multi-frequency impedance readouts as they allow zooming in on changes in membrane topology, cell-cell or cell-matrix junctions deconvoluting the complex whole cell response, for instance, to G-protein-coupled receptor (GPCRs) activation. Here, multi-frequency impedance measurements were used to monitor cell adhesion, cell specific structural changes, proliferation and cytotoxicity. The multi-frequency character of the data allows selecting the most sensitive frequency for a particular application or cell type after data acquisition is complete. Impedance spectra were calculated and data acquired with CardioExcyte96 impedance recording system, for HeLa, HX293 and CHO cells to demonstrate cell-type specific differences under stationary conditions. Cells covering the electrode impede the applied current and cell responses to experimental challenges will induce corresponding impedance changes. Frequency-dependent impedance data reveal individual drifts of the most sensitive frequency, i.e. of the maximal responses indicating cell type specific changes in cell junctions and proliferation dynamics. The expected concentration dependent toxicity was observed for Escin showing decreasing impedance values with exposure time and a reduction of the maximal responses to lower frequencies. GPCR-mediated signal transduction was investigated by applying the endogenous agonist in dose-response studies or receptor-independent agents that are used for pathway deconvolution. In conclusion, multi-frequency impedance data allow for a non-invasive continuous monitoring of cells with the option to zoom in on certain cell aspects or to tailor the readout to individual cell types for improved data interpretation.

3172 A Tubular Organoid-Derived Gut-on-a-Chip Model Suitable for Drug Development Research


Microfluidic techniques are increasingly recognized as an important toolbox to add physiologically relevant cues to traditional cell culture. These cues include long term stability and complex peristaltic flow. Microfluidic technology allows patterning of cell layers as stratified co-cultures that are devoid of artificial membranes, in order to capture complex tissue architectures found in vivo. Previously, we have introduced the OrganoPlate platform for growing human intestinal gut tubules in a membrane-free manner. Although suitable for toxicity studies, this model uses human intestinal cell lines, such as adenocarcinoma line Caco-2, which has limited differentiation capabilities and harbors gene mutations. In contrast, intestinal organoids are excellent surrogates of patients, capable of taking on the function of the tissue of origin. Combining adult human stem cell derived intestinal organoids with the microfluidic technology serves as a powerful platform to study physiology and disease mechanisms in patient specific gut models. We established a standardized tubular shaped epithelial barrier model of the intestinal tract showing rapid cell polarization, tight junction formation and proper expression of intestinal markers. OrganoPlate-grown miniaturized gut tubules are suitable for development of complex models to mimic in vivo metabolic and immunological responses through co-culture with vessels, immune cells and/or bacteria. These organoid-derived gut tubules are accessible from both apical and basolateral side and are suitable for high-throughput screening of compound effects. Complementing the OrganoPlate real-time imaging of barrier integrity, we developed a novel technique for on-a-Chip epithelial/endothelial tissue TEER investigation with very fast measurement times, automated signal extraction and compatible with tissue culture incubator environments. This provides a valuable, non-invasive tool for assessing barrier functions of healthy and diseases intestinal models. Protocols have been established that allow automated readout of the barrier integrity, followed by image analysis and quantification. Such tubular-shaped organoid-derived gut-on-a-chip platform can be used in an assay-ready manner to aid the drug development research.

3173 High Interlaboratory Reproducibility in Transfer of Duplex Sequencing Technology


Advances in Next Generation Sequencing (NGS) technology in the last decade have provided a powerful tool to detect mutations in tissues of any species in a high throughput manner. However, the high technical error rate of standard NGS has limited its application in measuring ultra-low somatic and germline mutations. By comparing reads derived from both original strands of DNA molecules, Duplex Sequencing (DS) technology achieves extremely high accuracy and it is possible to directly characterize the mutant frequency and mutation spectra in vehicle control and mutagen-treated animals. As a critical step in technology transfer, we report the comparison between DS mutagenesis assays that were performed independently by TwinStrand and MilliporeSigma. In both studies, the compound of Big Blue® F344 male rats with either N-ethyl-N-nitrosourea (ENU) or benzo[a]pyrene (B[a]P) induced obvious increases in the mutant frequency in liver. Significantly, near perfect correlation (R=0.99) was observed between MilliporeSigma and TwinStrand studies in both absolute mutant frequencies and fold-increase of mutant frequencies in treated groups compared with animals treated with vehicle control (olive oil, VC). The unsupervised hierarchical clustering of simple base substitution spectra shows very consistent clustering pattern in the corresponding samples of the two studies. The cosine similarity between trinucleotide spectra of samples from each group at MilliporeSigma and TwinStrand are in near perfect concordance. Furthermore, each treatment group displayed distinct trinucleotide spectra that are associated with different types of mutational signatures in the COSMIC database of human cancers. This high inter-lab reproducibility between TwinStrand and MilliporeSigma was also observed in rat bone marrow samples. Taken together, these results further demonstrated the power of DS to detect background and induced mutagenesis in different tissues and the interlaboratory reproducibility of the technology thereby providing evidence that the technology was successfully transferred to MilliporeSigma.

3174 Thermal Aerosolization of Chloroquine and Hydroxychloroquine and Pharmacokinetic Prediction of Inhaled Aerosols


In response to the coronavirus disease 2019 (COVID-19) pandemic of 2019-20, the pharmacological activity of existing drugs has been evaluated in vitro. Antimalarial drugs such as chloroquine (CQ) and hydroxychloroquine (HCQ) were found to be effective against SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) in vitro and were initially recommended for therapeutic and prophylactic treatment of COVID-19. Previous research has demonstrated a narrow margin between the therapeutic and toxic doses of CQ and HCQ. In this study, we developed inhalable formulations of CQ and HCQ. The drugs were solubilized in propylene glycol and subjected to thermolysis to generate an aerosol. The liquid formulation was evaporated by heating and subsequently cooled, which triggered nucleation and condensation processes that led to aerosol formation. The aerosol was transferred by using a programmable dual syringe pump (PDP), with a simulated inhalation regime of 55 mL delivered in a 3-s puff duration and 30-s interval. The PDP was attached to a secondary electrospray ionization (SUPER SESI) device interfaced with a Q Exactive HF mass spectrometer (MS) in order to detect the chemicals. Transfer rate was assessed by analyzing the chemicals present in the aerosol particles trapped on a Cambridge filter pad, by using liquid chromatography-mass spectrometry (LC-MS). The CQ and HCQ models were developed by integrating detailed species-specific respiratory tract physiology and lysosomal trapping parameters to predict the pharmacokinetics of inhaled aerosols. Thermal aerosols were generated from 40 mg mL\(^{-1}\) CQ and 100 mg mL\(^{-1}\) HCQ liquid formulations, which delivered 0.15 and 0.33 mg of the compound per puff, respectively. According to the model predictions, an inhalation dosing regimen of 10 puffs taken three times on day 1 and up to 3 puffs taken three times from day 2 onwards may yield therapeutically effective concentrations in the lungs. Inhalation of CQ and HCQ aerosols may maximize their concentrations in the lungs while minimizing systemic exposure.
Characterization of a Subcutaneous Injection of Tenofovir Alafenamide Long-Acting Formulations in Beagles

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Tenofovir alafenamide (TAF) long-acting injectable (LAI) formulations were evaluated in local tolerance studies via single subcutaneous injection to dogs to assess the reversibility, persistence, or delayed occurrence of local effects after a 4- or 8-week observation period. Four groups (6/sex/group) of beagle dogs were administered doses of placebo, TAF and/or Depo-Medrol® (a long acting formulation of methylprednisolone), at various concentrations and dosing schedules. Each dose group included negative, clinical observations, body weights, food consumption, dermal observations, clinical pathology and microscopic evaluation of dose sites and were conducted 4 and 8 weeks post dose. TAF injection site reactions (ISRs) of erythema and edema began to manifest on Day 2, and although they generally lessened in severity as the study progressed, some ISRs persisted until the end of the study. Ulcerated dose sites occurred in animals dosed ≥48 mg TAF starting on study day 8 and the severity of clinical dermal observations increased based on dose volume. Placebo control article and TAF-related macroscopic findings of skin thickening were observed at injection sites at terminal necropsies. At the 4-week necropsy, TAF-related microscopic findings were minimal to marked adipose tissue necrosis, mixed cell inflammation, and fibrosis (with or without a pseudocyst); slight mononuclear cell infiltrate; and/or minimal granulomatous inflammation. At the 8-week necropsy, TAF-related microscopic findings were generally similar to those seen at week 4 but were less severe and consistent with reversal after concurrent formulation(s) were used to evaluate if the inflammation could be mitigated. The overall lowest incidence and/or severity for these findings was noted for animals administered TAF with a high dose of Depo-Medrol®, and the highest incidence/severity was noted for animals administered TAF alone. Exposure (AUC0-168h, Cmax) to TAF and tenofovir (TFV) generally increased with increasing dose in a dose proportional manner. In 12 long-term studies, the studies used different routes of administration, species, and dosages. A single subcutaneous dose of TAF LAI is 24 mg TAF /dose site.

Validation of the SafeDesign Reporter Assay for the Quantification of the Onset of Adverse Responses and to Reveal the Underlying Mode of Action

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Classical chemical safety assessment often relies on simple cytotoxicity end-point measurements. However, to translate these results to the physiological relevant human situation still appears to be challenging. We therefore developed the SafeDesign platform, a unique new approach method (NAM) that can be applied to accurately quantify chemical-induced stress response pathway at single cell level to reveal the toxicological Mode of Action (MoA) of novel medicines, (agro)chemicals, cosmetics and food ingredients. The SafeDesign platform consists of a large collection of genetically engineered human liver HepG2 cell lines. Each cell line contains a green fluorescent protein reporter that accurately reflects a specific cellular stress response signal transduction pathway. To visualize activation of the different cellular stress response reporters, we developed an automated live cell imaging and data analysis pipeline to derive a unique quantitative toxicity fingerprint of chemicals. The advantage of this method is its use of a limited number of reporters (e.g. barbitaloxone methyl, cisplatin, tunicamycin, tumor necrosis factor-α) on reporter cell lines for oxidative stress, genetic stress, unfolded protein response and inflammation. To further increase the applicability of the GFP reporter assay, the HepG2 cell lines were differentiated into 3D spheroids. After 3 weeks of differentiation, the spheroids showed increased cytochrome P450 activity and were used to metabolite a collection of compounds that are highly relevant for drug-induced liver injury (DILI), e.g. trazodone, bicitralamide, trovafloxacin. Together, we developed a new mechanism-based reporter cell assay, SafeDesign, that can provide mechanistic toxicity information for early chemical safety testing, read-across, adverse outcome pathway (AOP) and weight-of-evidence (WoE) approaches.

Safety Assessment of Seven Degradants from Over-the-Counter (OTC) Drugs

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Many OTCs, such as oral antihistamines, have been on the commercial market since the 1940’s - well before testing methods and establishment of ICH/FDA guidelines for impurity assessment. Evaluating the safety of specific degradant levels (i.e., qualification) helps to assure consumer safety related to OTC drugs. The present study applies a stepwise, weight-of-evidence (WOE) approach which considers in silico modeling, empirical testing data, read-across, metabolic relation to the active pharmaceutical ingredient (API), or other methods specified in ICH/FDA guidelines to qualify seven degradants from three OTC drugs. Hazard characterization data were assessed by comparing to upper-end degradant exposures (i.e., maximum daily OTC intake, 10th percentile body weight) in the youngest population designated by the respective use in 21 CFR to qualitatively and quantitatively assess potential risk. As an example, assessments supported qualifications of up to 4% for 4,6-dihydroxy-2-methyliosquinoline (4,6-DMQ) and 4,8-dihydroxy-2-methyliosquinoline (4,8-DMQ) in phenylephrine (PE) based on three lines of evidence. For both compounds, there were a lack of genotoxicity structural alerts using both rules-based and statistical-based in silico modeling with Derek/Leadscope. Empirical data on 4,6-DMQ demonstrated a lack of both bacterial cell mutagenicity and mammalian cell clastogenicity. A quantitative risk assessment compared upper-end 4,6-DMQ exposures in young children to the no-observed-effect level (NOEL) from a 14-day gavage study in rats, resulting in margins of oral exposure ranging from >214 to >375, supporting safety. The empirical data and safety assessment from 4,6 DMQ were also applied to its isomer, 4,8 DMQ, in a read-across approach for establishing safety. Benzophenone was the most data-rich degradant evaluated and was qualified based on three different uses (e.g., as a sleep aid, antiemetic, or an anti-histamine), primarily via acceptable margins of safety based on permissible daily exposures (PDE) derived from long-term studies. A PDE-based approach was also applied for phenylephrine using read-across to PE due to the high degree of structural similarity. In some cases, such as chlorpheniramine-N-oxide, the degradant was also a metabolite of the API, contributing a line of evidence to the safety assessment. These data contribute to the utility of the stepwise, WOE approach to qualify OTC degradants for both data-poor and data-rich substances.
Drug-induced liver injury (DILI) remains a major concern for drug development programs, due to the risk of late stage clinical trial failures and post-marketing withdrawal. A variety of in vitro approaches have been used in an effort to improve prediction of DILI earlier in discovery, such as organotypic three-dimensional (3D) microtissue combined with High-Content Analysis (HCA). Mechanistic understanding of DILI can be gained by combining the most predictive and physiologically relevant in vitro models with analysis through high-throughput RNA sequencing to deliver more comprehensive toxicity profiles. Transcriptomics has been shown to play an important role in determining differentially expressed genes (DEGs), mechanisms of action and induced cell stress pathways associated with drug exposure. A pipeline has been established to fully automate screening compound libraries, a 128 reference drug library with and without clinical DILI compounds have been profiled across an eight dose response range at multiple time points including 14 day incubations. The models evaluated were HepaRG cells and primary human hepatocytes (PHH) in both 2D and as 3D organoids. The transcription profiles obtained allowed grouping of DILI positive and negative compounds into functional clusters by PCA (principal component analysis) and t-SNE analysis. DEGs in a dose-dependent manner were observed for DILI compounds and was shown to be mechanism and DILI rank dependent. Machine Learning combined with transcriptomic profiling of PHH allowed the identification of DILI compounds with 81% of sensitivity, 86% specificity and 82% accuracy. Also specific gene signatures could be associated with individual mechanisms of action. In summary, predictive toxicogenomics combined with organotypic models can be used to profile novel chemical entities to determine DILI risk, providing insight in to the potential mode of action implicated in the drugs toxicity.

3180 Species Specific Urothelial Toxicity with an Anti-HIV Non-catalytic Site Integrate Inhibitor (NCINI) Is Related to Unusual pH-Dependent Physicochemical Changes

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GS-9695 and GS-9822 are next generation Non-Catalytic Site Integrate Inhibitors (NCINIs) with significantly improved potency against HIV compared with previously developed drugs such as BI-224436. However, preclinical studies revealed bladder lesions in the cynomolgus monkey with both GS-9695 and GS-9822 that halted these two novel compounds in their development. The lesions were unlikely to be attributable to the target itself since NCINIs specifically target the viral insertion protein and no mammalian homologue is known. Secondary pharmacology studies did not offer any explanation for the lesions. GS-9822 compounds adversely affected mitochondrial respiration but there was no plausible explanation for the observed lack of an effect in the rat. Proteins implicated in cell-cell interactions and/or bladder integrity (e-cadherin, pan-cytokeratin, uroplakins) were analyzed in bladder but expression patterns did not provide any insight. Since the lesion was characterized by inflammation and disruption of the urothelial morphology, we investigated whether this could be attributable to physico-chemical changes in the bladder in the cynomolgus monkey (urinary pH 5.5-7.4) that might not occur in the bladder of rats (urinary pH 7.3-8.5). In measurements of surface activity, GS-9822 showed an unusual transition from a monolayer to a bilayer at the air/water interface with decreasing pH, attributed to the strong association between drug molecules in adjacent bilayer leaflets. Structural analysis of GS-9822 and GS-9695 showed zwiterionic characteristics over the range of pHs expected in cynomolgus monkey urine, a property expected to be highly disruptive to the urothelium. It seems unlikely that this exotic surface behaviour observed with GS-9822 (which causes bladder lesions) would have occurred with BI-224436 since BI-224436 would transition from neutral to cationic (never zwiterionic) with decreasing pH. These data provide useful insights for others that may be involved in current or future discovery and development of NCINIs and related compounds.

3182 Differences in Spontaneous Neoplastic Incidence in Crl:CD(SD) Rats and Crl:WI(Han) Rats

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Two rat strains often used in pharmaceutical development programs are the Charles River Crl:CD(SD) rat (SD rat) and the Crl:WI(Han) rat (WH rat). Previously published comparisons of spontaneous neoplasms in these two strains have relied on nonconcurrent databases obtained from different companies. In this presentation, the spontaneous incidences of major and minor neoplasms in each strain in 2-year carcinogenicity studies were compared from data compiled from multiple Charles River Safety Assessment facilities using studies that were completed from 2016 to 2020. The total number of each tissue examined for the two strains was at least 1000/sex per strain and at least 3030/sex for SD rats. In both strains, neoplasms that are frequently fatal include pituitary neoplasms in both sexes and mammary neoplasms in female rats; the incidence of pituitary adenomas and carcinomas in WH rats was 37% to 73% lower in each sex when compared to SD rats, and the incidence of mammary carcinomas and fibroadenomas was 75% and 56% (respectively) lower in WH rats when compared to SD rats. The incidences of various neoplasms in the brain, Harderian gland, kidney, liver, lung, ovary, and skin were generally comparable between the two strains. In the adrenal gland, the incidence of pheochromocytomas (benign or malignant) in SD males and females ranged from 2-fold to nearly 5-fold that of WH rats, whereas the incidence of cortical cell adenomas in male WH rats was at least 2-fold of that found in male SD rats. In the thyroid gland, while C-cell adenoma incidence was comparable between the two strains in both sexes, follicular cell adenomas in WH males and females were more than 3-fold and follicular cell carcinomas were nearly 2-fold that observed in the SD strain. Other tissues in which the neoplastic incidences were at least 2-fold greater in WH males and/or females when compared to SD rats included the mesenteric lymph node (hemangio- mas in both sexes and hemangiosarcomas in males), the thymus (benign and malignant thymomas), and uterus (endometrial adenomas, adenocarcinomas, stromal polyps, and stromal sarcomas). In the pancreas, acinar and islet cell neoplasms in SD males and females were at least 2-fold of that observed in WH males and females. A better understanding of these strain differences in neoplastic incidence will assist in selection of the most appropriate rat strain for drug safety assessment programs.
3183 Reproducibility of Survival and Spontaneous and NMU-Induced Neoplastic Incidence in CByB6F1-Tg(HRAS)2Jic (Tg.rash2) Mice over the Last Decade

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The CByB6F1-Tg(HRAS)2Jic (Tg.rash2) model is the most common 6-month carcinogenicity assay used as an alternative to the standard 2-year mouse carcinogenicity assay. In this presentation, the survival, spontaneous incidence of neoplasms, and NMU-induced neoplastic incidence in Tg.rash1 mice was compared from data compiled from a single Charles River Safety Assessment facility of a total of 26 studies that were conducted from 2011 to 2020. In these studies, N-nitro-N-methylurea (NMU) obtained from the same vendor was used as the positive control. The total numbers of negative (vehicle or untreated) controls for each sex over the ten-year period were 1029 (males) and 929 (females), and the total numbers of positive controls were 395 (males) and 380 (females). From 2011 to 2015 and from 2016 to 2020, the percent overall survival to Week 7 for negative control males was 96.0% and 96.0%, and for female negative control females, 95.6% and 96.2%, respectively. The overall average percent of negative controls with neoplasms from 2011 to 2015 was 19.2% for males and 22.0% for females; the overall average percent of negative controls with neoplasms from 2016 to 2020 was 19.4% for males and 20.2% for females. Major spontaneous neoplasms in both male and female negative control mice included bronchioalveolar adenomas/carcinomas as well as splenic and non-splenic hemangiosarcomas. Nearby all NMU-treated positive control mice developed neoplasms; from 2011 to 2015 the neoplastic incidence was 97.8% for males and 98.9% for females, and from 2016 to 2020, the neoplastic incidence was 98.4% for males and 97.6% for females. As expected, the overall survival of positive control mice to Week 27, was low, ranging from 12.2% to 16.4% depending on the sex and 5-year timeframe. The two major neoplasms observed in positive controls were lymphomas (70.0% in males and 78.9% in females from 2011-2015; 63.9% in males and 70.7% in females from 2016-2020) and papillomas/squamous cell carcinomas of the stomach (91.1% in males and 75.6% in females from 2011-2015; 87.5% in males and 77.6% in females from 2016-2020). These data demonstrate that the survival and neoplastic incidence in negative and positive control Tg.rash2 mice were reproducible in our laboratory over the past ten years.

3184 Retrospective Analysis on the Tolerability of Commonly Used Vehicles in Nonclinical Studies


Vehicles, commonly used for the formulation of Test Articles in non-clinical studies, can induce significant clinical signs or toxic effects that might complicate the study results interpretation and the evaluation of the potential toxicity of the active substance being tested while also deteriorating animals’ health. We have performed a retrospective analysis on vehicles used over the past 7 years in studies conducted in rats, dogs, Cynomolgus monkeys and minipigs. The most commonly used vehicles were evaluated and included cyclodextrins (HPBCD and SBECDD), polyethylene glycol (PEG) 400, methyl cellulose, carboxymethylcellulose, dimethyl sulfoxide and tween 80, used to solubilize or stabilize the active substance or as thickening agents. The vehicle-related effects on in-life observations and postmortem examination were analyzed from general toxicology studies, from Dose Range Finding to 39 Week studies. The effects were compiled depending on the vehicle quantity, the route of administration and species.Intravenous administration of both cyclodextrins resulted in vacuolation in the kidneys and urinary bladder, histiocytosis in the lungs as well as inflammation and hemorrhage at the injection site in almost all studies conducted in rats, dogs and Cynomolgus monkeys. When administered intravenously in rats and Cynomolgus monkeys and subcutaneously in minipigs, inflammation and necrosis at the injection site were occasionally observed. After oral administration of PEG 400, hypersalivation and abnormal foraging in rats, Cynomolgus monkeys and minipigs. After injection of PEG 400, intravenously in rats and Cynomolgus monkeys and subcutaneously in minipigs, inflammation and necrosis at the injection site were noted. Abnormal vacuolation of the liver was occasionally observed. After oral administration of PEG 400, hypersalivation and abnormal foraging, soft and/or colored feces were observed in some of the rats, dog and Cynomolgus monkey studies. The use of methylcellulose and tween 80 resulted in rare clinical signs, respectively salivation and ploughing following oral administration in rats and inflammation and necrosis at the injection site following subcutaneous administration in minipigs. Carbaryl administered orally, was not associated with any relevant finding. This retrospective analysis brings valuable information in our toxicology studies, such as acceptable ranges for the use of vehicles in different species. Knowledge on the findings induced by the vehicles allows us to better understand and distinguish these effects from those associated with Test Articles.

3185 A Combined In Vitro Approach for the Dual Detection of Functional and Structural Cardiotoxicity

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Cardiotoxicity is a major cause of drug attrition during pre-clinical and clinical drug development. Drugs can exhibit functional changes defined as an acute alteration in the mechanical function of the myocardium or structural in nature as defined by morphological damage to cardiomyocytes and/or loss of viability. In recent years in vitro strategies have been developed to allow the high throughput assessment of functional cardiotoxicity changes through kinetic monitoring of calcium transients, while structural morphology can be monitored in a high throughput manner using high content imaging (HCI). Here we describe a combined method to allow the morphological analysis of cardiomyocytes alongside calcium transient assessment in order to better define functional and structural cardiotoxicity events. Human induced pluripotent cardiomyocytes (hiPSC-CMs) are seeded in 384 well plates for a minimum of 10 days before incubation with EarlyTox Cardiotoxicity (Molecular Devices) fluorescent dye. Following a 2 hour incubation, compound is applied at 8 concentrations in triplicate utilising a compound set comprising structural and functional cardiotoxins alongside dual toxicity compounds and non-cardiotoxins (total of 14 compounds). Fast kinetic fluorescent reading is then performed on a Cytation 3 Cell Imaging Multi-Mode Reader (BioTek). High content imaging of calcium homeostasis (EarlyTox) and mitochondrial function (TMRE) is then performed using an ArrayScan XTI H1 reader (ThermoScientific). Finally, the compound is applied for the quantification of extracellular ATP using CellTiter-Glo (Promega). This combined assay approach allows the detection of acute functional cardiotoxicity; epinephrine and isoproterenol increase calcium transient peak frequency (MCC; 0.006 µM & 0.005 µM, respectively) while verapamil decreases peak frequency and amplitude (MCC; 0.03 µM & 0.02 µM, respectively). Early signs of morphological changes can also be detected; sunitinib is a dual toxicity compound (functional and structural) which exhibits a decrease in peak calcium transient amplitude (MCC; 88 µM) alongside cell morphology changes (MCC; 144 µM). This study shows a combined cellular assessment strategy can improve the in vitro to in vivo translation and risk assessment of the potential for novel compounds to elicit functional and structural cardiotoxic events.
Prior to initiation of clinical trials, repeat-dose toxicity studies are conducted in multiple species to support the safety of the active pharmaceutical ingredient (API) in the proposed clinical dosing regimen, route of administration, and duration of treatment. The primary metric used to extrapolate the safety of clinical dosage from repeat-dose toxicity study results is the safety margin, i.e. the ratio of no observable adverse effect level (NOAEL) from the toxicology study to the proposed clinical dose. This ratio can be calculated by using allometric scaling to approximate the equivalent human dose from that used in the toxicology study based on the body surface area of the species employed or by comparing the empirically measured maximum plasma concentration (Cmax) or total plasma exposure (AUC) between the toxicokinetic animal data and the human pharmacokinetic data, if available. Another important consideration in drug safety evaluation is the nature and severity of the toxicities observed at doses above the NOAEL. As toxicity studies of various durations are typically conducted in multiple species and potentially via multiple routes of administration, it can be challenging to effectively integrate all of this information. In collaboration with the Pharmaceutical Users Software Exchange (PHUSE) Nonclinical Scripts Working Group and with consultation from toxicologists at FDA, an open source software application was developed to allow users to interactively visualize safety margins and the severity of user-defined significant toxicities across studies throughout a drug development program in a single figure. The application can also present this information in tabular form that can be exported in various formats, e.g. CSV, Excel or Word files. These functionalities are designed to facilitate holistic evaluation of the drug safety by generating graphical and tabular summaries of the full toxicological profile of an API.

Seizure liability is amongst the most frequent drug-induced neurological adverse effect. EEG monitoring is commonly used to investigate seizurogenic potential but also to assess neuromodulatory effects. Telemetry is considered as the gold standard for non-clinical models with a recording frequency typically set at 255 Hz or above. It is common practice to include multiple EEG derivations to monitor different brain regions in preclinical neurotoxicology studies and the dataset generated with such montage creates a real interpretation challenge. We reviewed EEG analysis strategies including a combination of automated algorithm driven detection, manual morphology review and qEEG. EEG data from rats, dogs, minipigs and non-human primates was included in the retrospective analysis. Results were comparable in all species and continuous clinical sign monitoring using video tended to increase the sensitivity of the seizurogenic effect detection. Manual EEG review at times when relevant neurological clinical signs were observed showed the highest correlation to abnormal EEG activity including inter-ictal epileptiform activity and seizures. Automated algorithm driven EEG detection showed high sensitivity for detection of seizures but had low specificity and did not successfully identify inter-ictal epileptiform activity. qEEG was a relevant biomarker preceding seizures for some drugs that were administered by parenteral routes but most drug administered by the oral route rarely induced significant qEEG changes prior to seizure onset. qEEG analysis was significantly improved by using time and frequency matched delta evaluations and taking into account potential sequence effects. In summary, EEG interpretation is a challenging task given the volume of data acquired in neurotoxicology studies using telemetry and multiple process analysis maybe the most suited strategy to identify adverse neurological effects and translation considerations.
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